ROOT-SHOOT ANATOMY AND POST-HARVEST VEGETATIVE CLONAL DEVELOPMENT IN *LOPHOPHORA WILLIAMSII* (CACTACEAE: CACTEAE): IMPLICATIONS FOR CONSERVATION

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ABSTRACT

Over the last four decades, the size and density of populations of *Lophophora williamsii* (peyote) have diminished markedly in large areas of South Texas where licensed peyote distributors harvest the cactus for ceremonial use by the Native American Church. Part of the problem lies in the fact that some harvesters are cutting plants too low on the subterranean stem or taproot. That practice precludes the regeneration of new stems and ultimately results in the death of the decapitated plants. To address this problem, we describe the anatomical distinctions between subterranean stem and root in *L. williamsii* as follows: The stem cortex can be distinguished by the cortical bundles running through the parenchyma, in contrast to the root cortex, which consists of pure parenchyma without cortical bundles. The pith at the center of the stem is pure parenchyma (without xylem) and is readily distinguished from the dilatated metaxylem (with masses of dark-staining metaxylem tracheary elements) occupying the center of the root. With these new anatomical tools, it is now possible to set up titration experiments, first in the greenhouse and then in the field, to generate practical biometric data to determine the maximum depth at which the peyote harvesters can cut the plants without significantly reducing the survival rate of the rootstocks left in the ground after harvest.

RESUMEN

Durante las últimas cuatro décadas, el tamaño y densidad de las poblaciones de *Lophophora wiilliamsii* (peyote) han disminuido drásticamente en grandes extensiones del sur de Texas donde se dan permisos a distribuidores de peyote para recolectar especimenes de uso en las ceremonias religiosas de la Native American Church. Parte del problema estriba en el hecho de que algunos colectores están cortando plantas muy abajo, o en el tallo subterráneo o en la raíz pivotante. Esta práctica impide la regeneración de nuevos tallos y finalmente ocasiona la muerte de las plantas decapitadas. Para abordar este problema, nosotros describimos las características anatómicas que distinguen el tallo subterráneo y la raíz de *L. williamsii* como sigue: El tejido cortical del tallo se distingue por la presencia de haces corticales que se distribuyen por el parénquima, atributo que contrasta con el tejido cortical de la raíz, el cual consiste en puro parénquima sin haces corticales. En el centro del tallo la médula es puro parénquima (sin xilema), y se reconoce claramente del metaxilema dilatado (parches de elementos traqueales del metaxilema teñidos de oscuro) que ocupa el centro de la raíz. En base a estas características anatómicas será posible hacer experimentos de corte, primero en invernadero y después en el campo, con la finalidad de generar datos que permitan determinar la profundidad máxima a la cual los recolectores de peyote pueden cortar las plantas sin reducir significativamente la supervivencia de los individuos que quedan en el suelo después de la colecta.

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INTRODUCTION

Peyote harvest as a conservation problem

Lophophora williamsii (Lem. ex Salm-Dyck) J.M. Coult. 1894, commonly called peyote, is a small, napiform, spineless cactus native to the Chihuahuan Desert and the Tamaulipecan Thornscrub of northeastern Mexico and adjacent Texas. While the mescaline-containing stems are ingested in various forms for their psychotropic effects, this behavior was generally proscribed by federal drug legislation in 1970. However, federal law provides protection for the use of peyote for bona fide religious ceremonial purposes by members of the Native American Church. The supply of peyote for such purposes is regulated by the Drug Enforcement Administration and the Texas Department of Public Safety. The regulated commerce in peyote begins with the harvest of peyote from wild populations by licensed peyote distributors or their agents. Commercial quantities of peyote occur in the U.S. only in Starr, Zapata, Webb and Jim Hogg Counties in South Texas, within 70 km of the Rio Grande, and all four currently licensed peyote distributors are based where peyote grows in those counties. Historically the peyote distributors have gained access to harvestable populations of peyote through peyote-specific lease agreements with private landowners. Most of the actual harvesting of peyote is done by contract laborers who are paid in accordance with the number and size of freshly cut "buttons" (tops of stems) of peyote that they deliver to the licensed distributors.

There has been a decrease in the number, size, extent and density of peyote populations in South Texas (Anderson 1995; Moreno 2005) over the past four decades. Much of this reduction in peyote numbers can be attributed to habitat destruction associated with urban development and agricultural practices such as rootplowing of native brush, and some adverse effects on population numbers may have been due to illicit harvesting. Such phenomena are difficult to quantify and virtually impossible to prevent. One factor that *is* quantifiable in the decline of peyote in South Texas is the regulated commercial harvest of peyote by the licensed distributors for ceremonial use by the Native American Church. Approximately two million peyote buttons per year have been harvested by these distributors over the last two decades (Texas Department of Public Safety, unpublished data). And the technique used in the harvest of those plants could logically have a substantial impact on the observed decline—or the potential recovery—of peyote populations in the Tamaulipecan Thornscrub of South Texas.

The proper technique for harvesting peyote is that the crown (i.e., the aerial, photosynthetic portion of the stem) of the peyote cactus is cut off at or immediately below its base, and the subterranean portion of the plant, including all or most of the subterranean portion of the stem, is left in the ground to regenerate one or more new crowns. Such harvesting of the commercially valuable crown of the cactus may be accomplished by cutting through the plant transversely at the level of the surface of the ground, at or near the interface of the green crown and the brown subterranean portion of the stem, using a machete, a cutting tool with a broad flat blade and a handle about 60 cm long (such as a hand edger), or virtually any kind of knife (M. Terry, pers. obs., Fig. 1a–c).

When proper harvesting technique is adhered to, the decapitated subterranean portion of the stem and the more distal taproot of the cactus (Fig. 2) remain intact and *in situ*, where the viable subterranean stem tissue will normally begin to regenerate one or more new crowns by lateral branching from axillary (areolar) buds within a few months after loss of the apical meristem (Fig. 3). Such regenerated crowns may in turn be sustainably harvested after they reach maturity, some years later (Fig. 4).

Peyote harvest—degeneration and death of the decapitated plant

Not every peyote plant responds in the same manner to removal of its apical meristem along with the crown at harvest. The simplest response is that described above: regeneration of one or more new crowns by lateral branching from (usually subterranean) stem tissue (Fig. 3). But frequently we observe a more complex, gradual response, which begins with simple lateral branching from the remaining stem of the decapitated plant. The difference is that the new stem branches, instead of remaining dependent on the taproot of the original plant, put down their own adventitious taproots and eventually become independent plants that detach themselves from the original plant, which—if its crown was completely removed at harvest—degenerates and dies in this process.

The unusual aspect of this second type of response of peyote to removal of its apical meristem in the harvesting process, is that the regeneration of new stems and the generation of new adventitious taproots from the new stem branches proceed in a seamless developmental process until at some point it must be recognized that the new, increasingly autonomous shoot-root units have become independent vegetative clones of the original plant. That is to say, where we started with a single decapitated plant undergoing development of new stem branches, we end up with what must be recognized as a moribund *parent* plant with viable clonal *progeny*.

Another remarkable feature of this process is that as the clonal progeny become larger and more nutritionally independent, the stem tissue connection between the parent plant and each of the vegetative clones degenerates to a slender tube consisting mostly of vascular tissue, while the parent plant (which has no photosynthetic capabilities of its own) steadily decreases in size and density as the nutrients stored in its parenchymal tissues are depleted and utilized by the growing clonal offspring. In the final phase of the process, the connection between the parent plant and the now nutritionally independent progeny disintegrates, and what is left of the decapitated parent plant dies. This series of events is depicted in Figures 5–12. All the photos in these figures are of

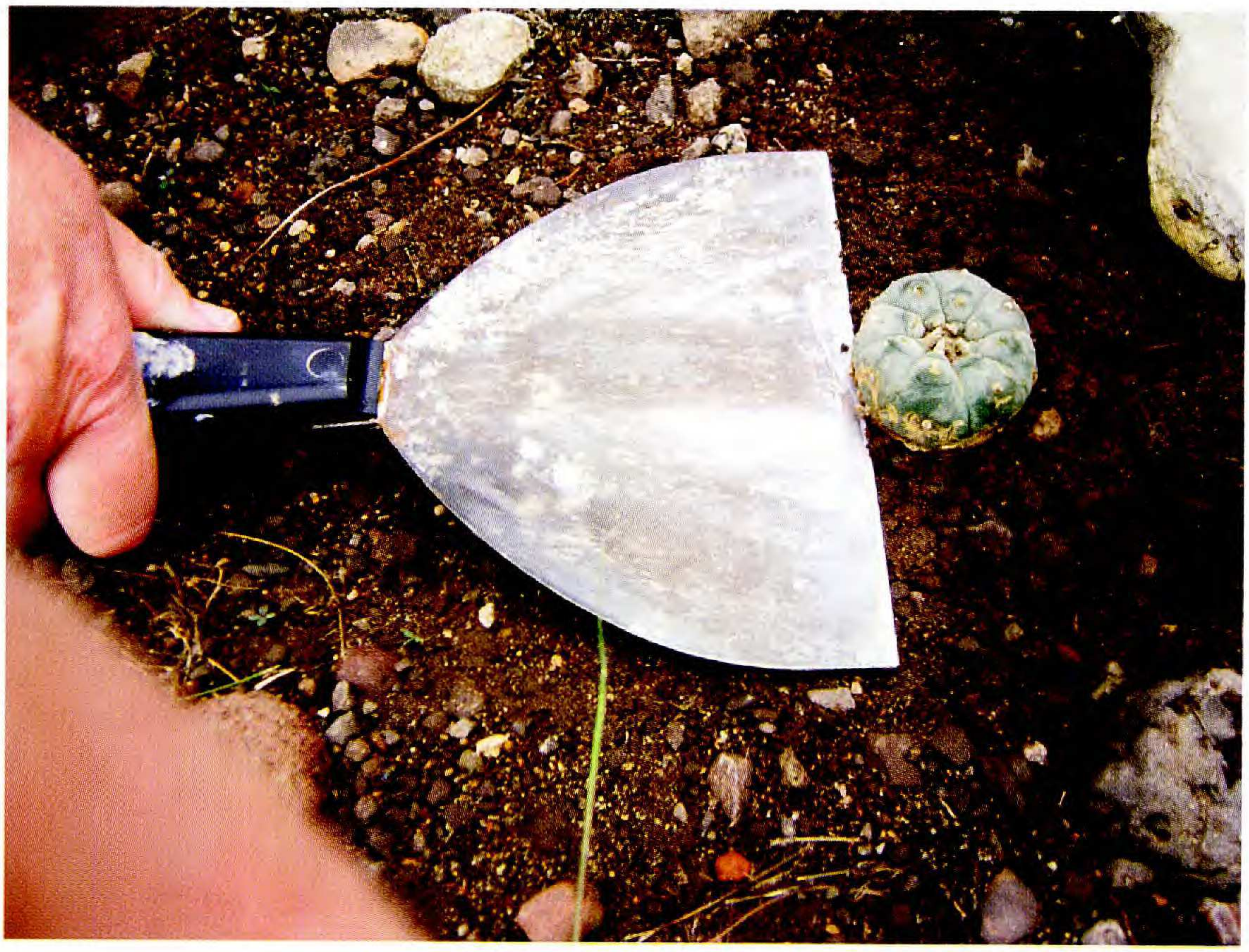

Fig. 1a. Proper peyote harvesting technique. Plant is cut transversely at base of crown, i.e., at ground level.

specimens that the first author collected from populations near Rio Grande City, Starr County, Texas.

This natural process of vegetative clone production in response to the excision of the apical meristem that occurs when peyote crowns are harvested, is remarkably congruent with the folk belief that harvesting peyote results in an increase in the number of plants in a population, and that where one plant grew before harvesting, several plants may be found after allowing an adequate period of time for the peyote to "grow back." A necessary condition for this process to occur, however, is that the harvesting be done in a manner that does not preclude the production of new stems from the subterranean stem of a plant from which the crown has been harvested. What does this mean in terms that could serve as a practical guideline for sustainable harvesting?

The answer must be based on an understanding of the anatomy of the root and stem of *Lophophora williamsii*. We begin with the observation that of all the people working with this plant—and here we include peyote distributors, members of the Native American Church, cactus hobbyists in countries where peyote cultivation is legal, and even botanists—very few appreciate the distinction between true root and subterranean stem. Yet this distinction is crucial to

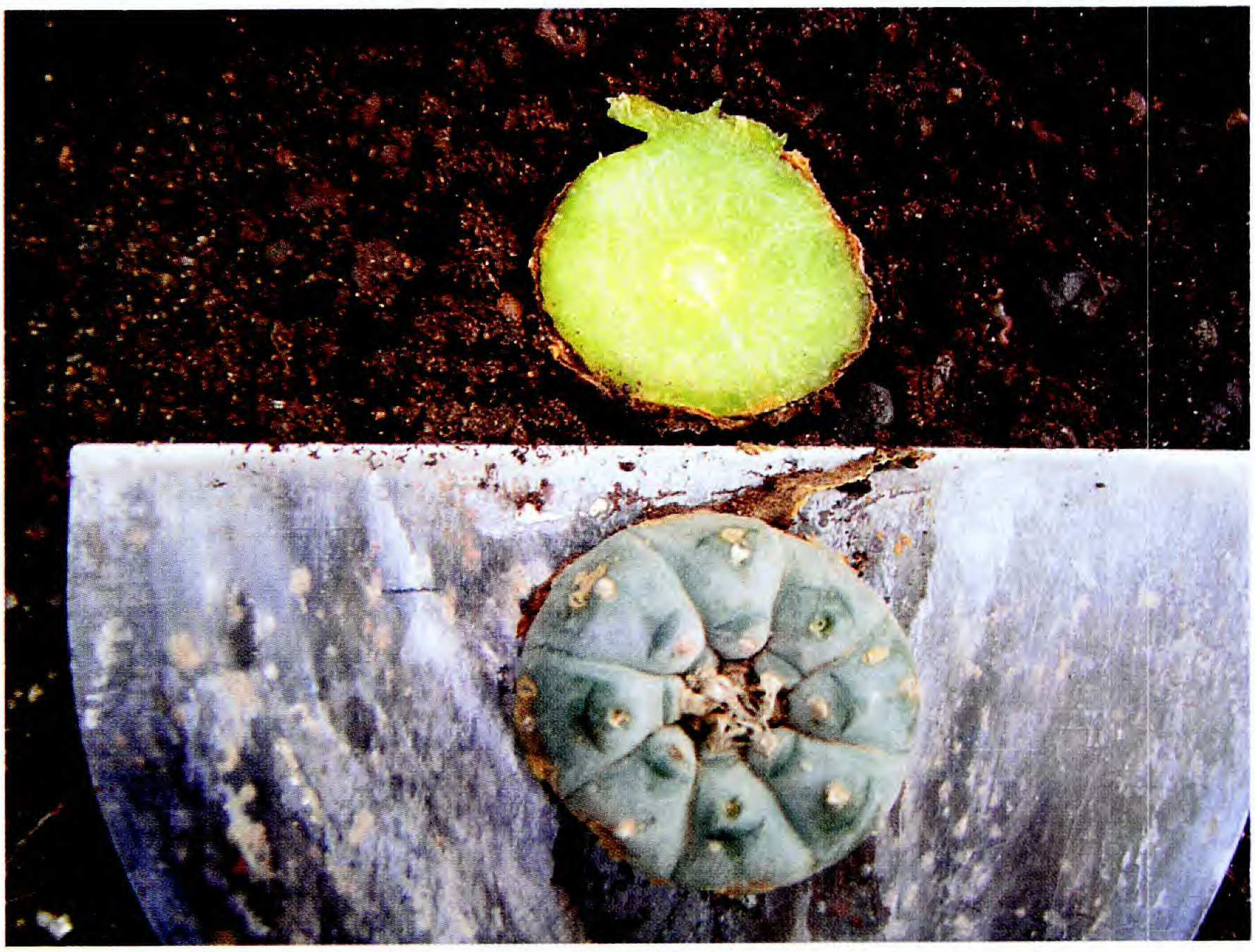

Fig. 1b Proper peyote harvesting technique. Cut has been made parallel to ground surface, and harvested crown (below, about 5 cm in diameter, with 8 ribs) has been removed from subterranean portion of stem (above, remaining in the ground). Cut surface shows cross section of vascular cylinder (ring of yellow tissue near center of stem), pith within the vascular cylinder, and yellowish green parenchymal tissue of the cortex of the stem (between vascular ring and epidermis).

an understanding of how to harvest peyote so that it will "grow back," because, as far as we have been able to determine from observations to date, new stem branches will develop only from stem tissue, not from root tissue. Therefore, if in harvesting the crown one cuts so deeply below the crown that all or most of the subterranean stem tissue is removed along with the crown, then there will be no possibility of new stem development, and the stemless root left in the ground will simply perish. With that as the operating premise, let us now consider the anatomy of the root and the shoot of *L. williamsii*, and how to distinguish between them.

Ideally we should like to be able to identify the shoot-root transition zone in order that at least some portion of the shoot should remain with the root of the plant left in the ground after harvest. Unfortunately, the plants normally protrude only 1–3 cm above the surface of the ground and have a large subterranean shoot that tapers gradually until it ends in a taproot. The shoot-root transition zone does not occur near the soil level where the seed germinated

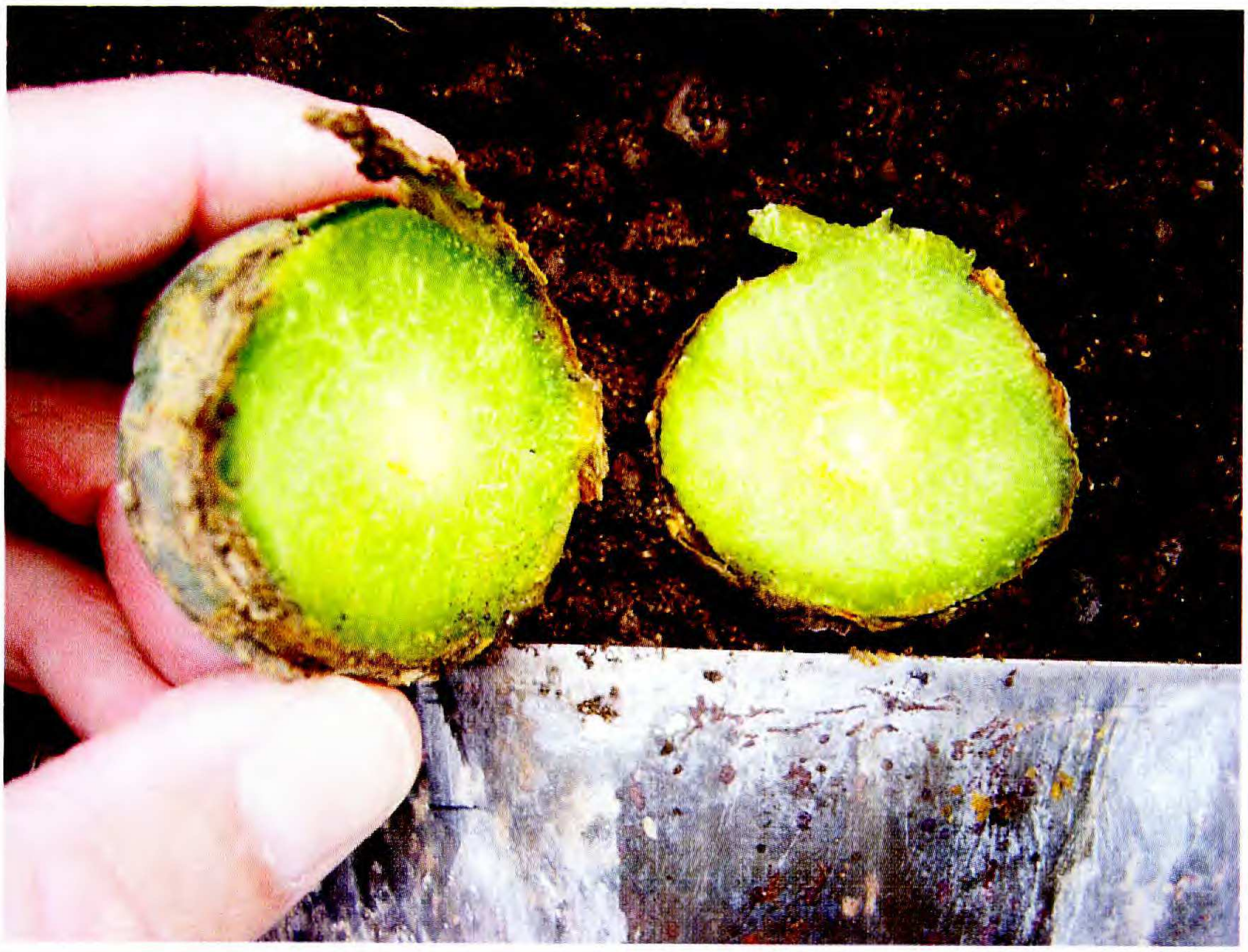

Fig. 1c. Proper peyote harvesting technique. Underside of harvested crown (left) shows very narrow (2–3 mm wide) ring of bark at perimeter of cut surface, indicating that the cut was barely below base of crown, in uppermost portion of subterranean stem. Cut surfaces match, except that crown parenchyma (left) is slightly greener due to higher chlorophyll content, whereas subterranean stem parenchyma (right) is more yellowish.

but instead occurs at various depths (higher in smaller plants, lower in larger plants). It is not possible to identify the shoot/root transition zone merely by examining an intact plant with the naked eye.

In most seed plants, young shoots and roots can be distinguished from each other because the shoot has a pith and cortex whereas the root lacks both these structures (Mauseth 1988). However, several other cacti grow like peyote—having a large below-ground shoot that tapers into a large taproot—and the shoot-root nature of those structures has not been clarified (Stone-Palmquist & Mauseth 2002). Early workers on the anatomy of *Lophophora* (Rouhier 1927; Bravo 1931; Janot & Bernier 1933) gave fair to very good anatomical descriptions of root and shoot, but even Bravo, whose description of the subterranean stem and root of *Lophophora* was the best available in its day, admitted that "[it is most difficult to know in which region the stem ends and in which the root begins]..." (Bravo 1931, translated from the Spanish). Because better harvest techniques may aid the survival of this species in areas subject to intensive harvesting for human consumption, as in South Texas, we undertook a histologic study of the anatomy of roots

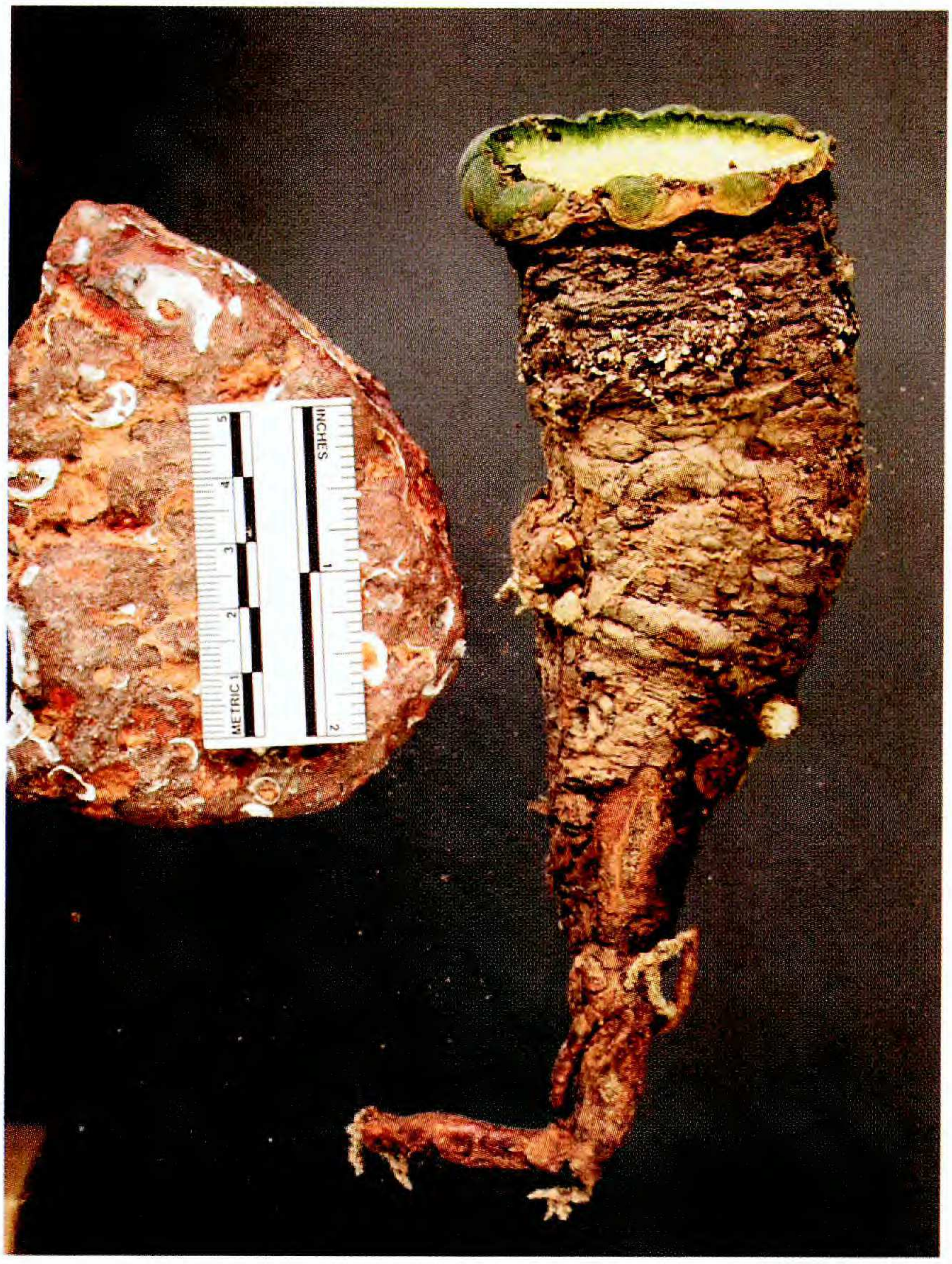

Fig. 2. Peyote plant immediately after most of crown has been harvested, showing cut just above base of harvested crown (green tissue at top), subterranean stem (brown bark-covered tissue immediately below cut base of crown) capable of regenerating new crown(s), and tapering taproot (bottom part of plant with a few visible lateral roots).

and shoots in *L. williamsii* to determine if there are reliable criteria for distinguishing the root from the shoot (and particularly the subterranean portion of the shoot). We especially looked for criteria that could be used in the field.

MATERIALS AND METHODS

Plants were collected by M. Terry (DEA Researchers Registration No. RT0269591) from a wild population of *L. williamsii* near Rio Grande City in Starr County, Texas, or, in the case of one specimen (the very large plant), donated for research purposes by law enforcement personnel. Specimens examined included two very small plants, two plants of intermediate size and one very large plant (Table 1).

TABLE 1. Dimensions of the three adult plants studied, listed from shortest to longest. All values are in millimeters. Shoots of plants #1212 and #1213 were sampled at ground level and at two below-ground levels; shoot of plant #1214 was sampled at only one below-ground level. The root of each plant was sampled only at the top of the root, where it most resembled a portion of shoot.

Sample	Plant length	Aerial height	Plant diam. at soil level	Shoot cortex thickness	Shoot pith diam.	Root cortex-like zone thickness	Root pith-like region diam.
Plant 1212, at ground level	135	24	55	20	4		
1212, at -20 mm				13	6		
1212, at -25 mm				9	6		
1212, root, -35 mm						1.5	10
Plant 1214, at ground level	140	44	78	32	10		
1214, at -35 mm				6	27		
1214, root, -41mm						3.0	27
Plant 1213, at ground level	160	40	59	14	6		
1213, at -20 mm				14	6		
1213, at -35 mm				9	8		
1213, root, -45 mm						1.0	3.4

Plants were dissected with care being taken to obtain samples of material that was definitely root (provisionally defined as the region below the uppermost point at which a lateral root had emerged), definitely shoot (namely the aerial portion of the shoot—specifically known as the crown, *sensu* Schultes (1938)—which has a blue-gray to blue-green epidermis, photosynthetic tissue and axillary buds), and definitely hypocotyl (the transition zone between shoot and root) samples being obtained by taking numerous samples between obvious root and obvious shoot). In all but the smallest, youngest plants, the plant material that was easily recognizable as root was located at least 35 mm below soil level (45 mm below in plant #1213; Table 1). Since the nature of the higher subterranean portions of *L. williamsii* was in question, samples were taken from all plants examined, with the position of each sample being carefully measured with the soil level (taken as the level of the base of the crown) as reference.

As tissue samples were obtained during dissection, they were immediately immersed in Navashin's solution, then aspirated in a vacuum chamber to remove air and permit rapid penetration of fixative. Tissues were fixed for 24 hours, dehydrated through mixtures of ethanol and tertiary butanol, then embedded in Paraplast Plus. After microtoming, sections were stained with Safranin and Fast Green by a procedure designed especially for cacti (Mauseth et al. 1985).

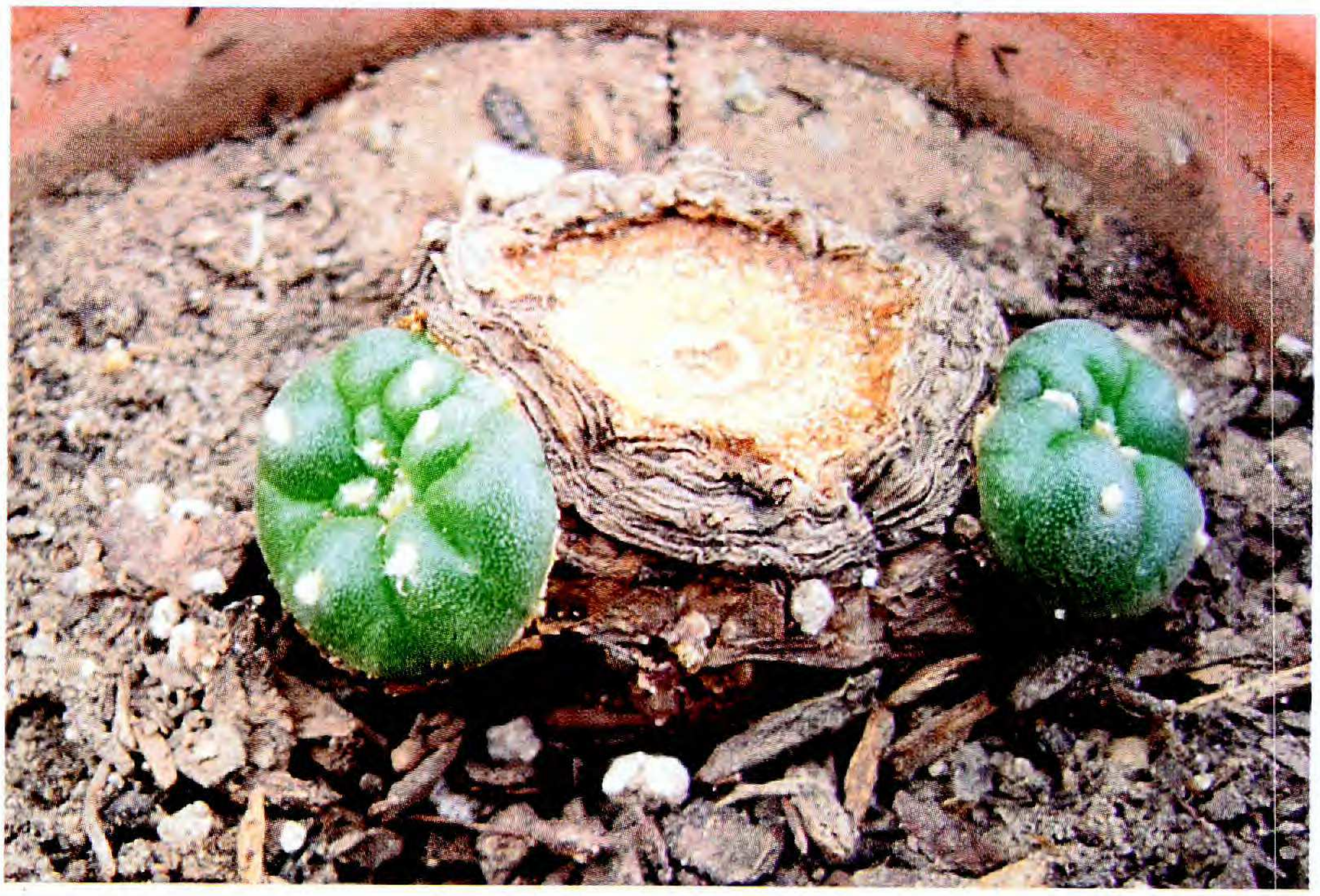

FIG. 3. Two young peyote crowns ("pups") regenerating by lateral branching from the upper edge of the subterranean stem of a plant that was decapitated 7.5 months previously. This plant is the same individual shown in Figs. 1a–1c. Each of the new crowns is ca. 1.5 cm in diameter

RESULTS

Morphology

All plants tapered gradually from an unbranched aerial shoot to a region of subterranean shoot, then to hypocotyl, and finally to taproot. The taper was uniform in most plants, without any abrupt change in diameter that might indicate the boundary between shoot and hypocotyl or between hypocotyl and root. The two smallest plants that we examined (whose above-ground, photosynthetic crowns were 15 mm in diameter) were only 31mm long (9 mm above ground, 22 mm below ground) and 50 mm long (10 mm above ground), so seeds must have germinated at or slightly below ground level. However, in the three adults we examined, the root/shoot junction was located at least 35 mm below the soil level (45 mm below in plant #1213), so plants of *Lophophora williamsii* must have contractile roots pulling the root/shoot junction deeper as the plant ages. All above-ground portions of shoot were covered with a blue epidermis; all subterranean portions were covered with thin, flaking brown bark. Ribs and axillary buds (often called areoles in cacti) were obvious on all above-ground portions, and withered areoles were occasionally detected (as much as 17 mm below soil level on plant #1213).

Slender lateral roots (1–3 mm diameter) emerged from taproots, but were extremely sparse, with only two or three present on any plant. This could be in

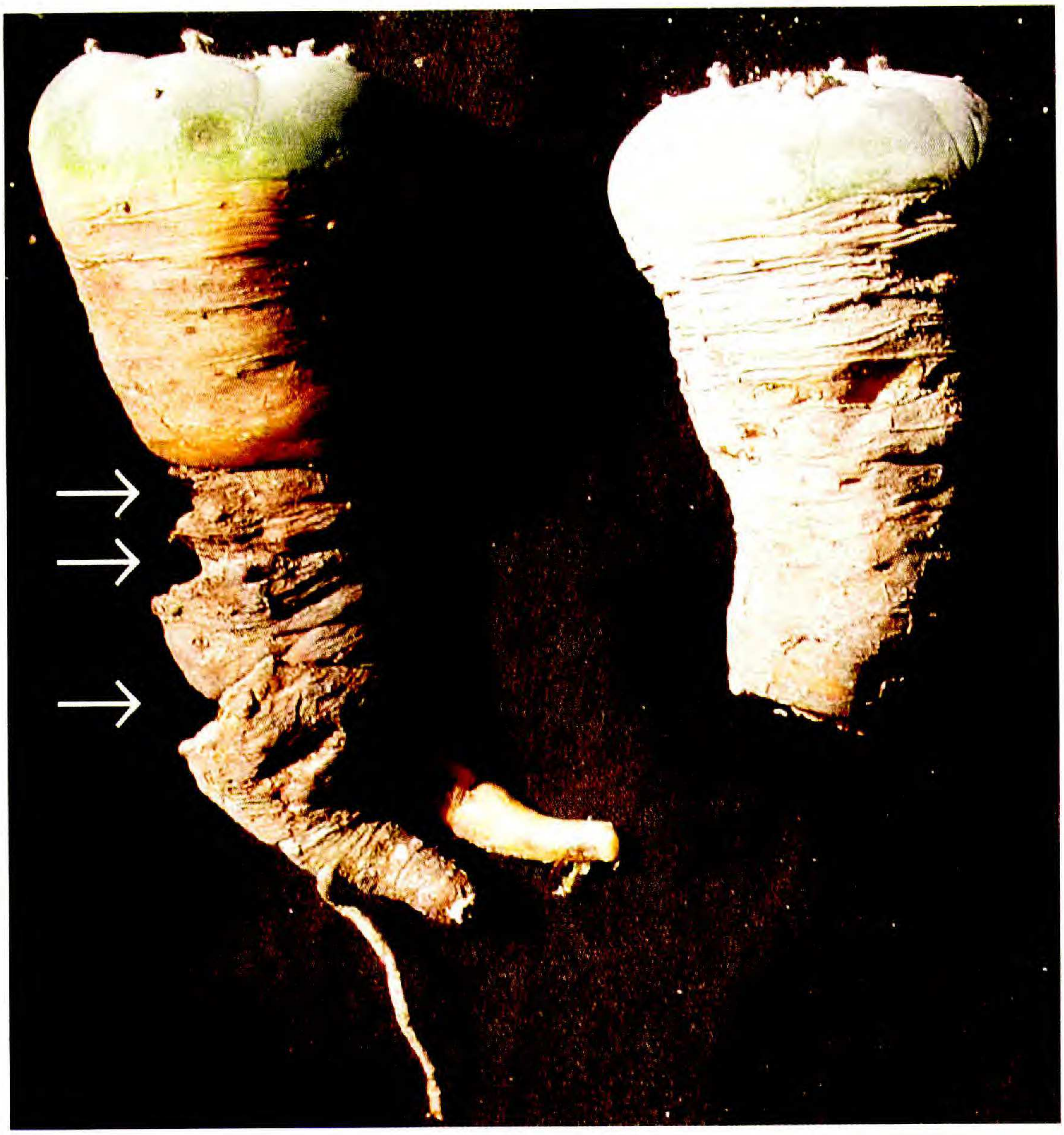

FIG. 4. Repeated harvesting of successive crowns from the same peyote plant. Individual on left bears notches (see arrows) indicating stem regeneration after having its crown harvested three times in the past. Individual on right has never had its crown harvested (the distal end of the root broke off when the plant was dug up for examination).

part because most lateral roots in this species are ephemeral (emerging in response to moisture, then being shed in conditions of drought), small, and fragile (M. Terry, pers. obs.). The result is that all but the few largest lateral roots are broken off and left in the ground when one uproots the plant from its natural growth site in habitat—no matter how carefully one goes about extracting the plant. When one grows the plant in loose, friable soil under artificial conditions, more lateral roots and their finer branches remain intact when the plant is depotted. The plants used in this study, however, were recently uprooted from

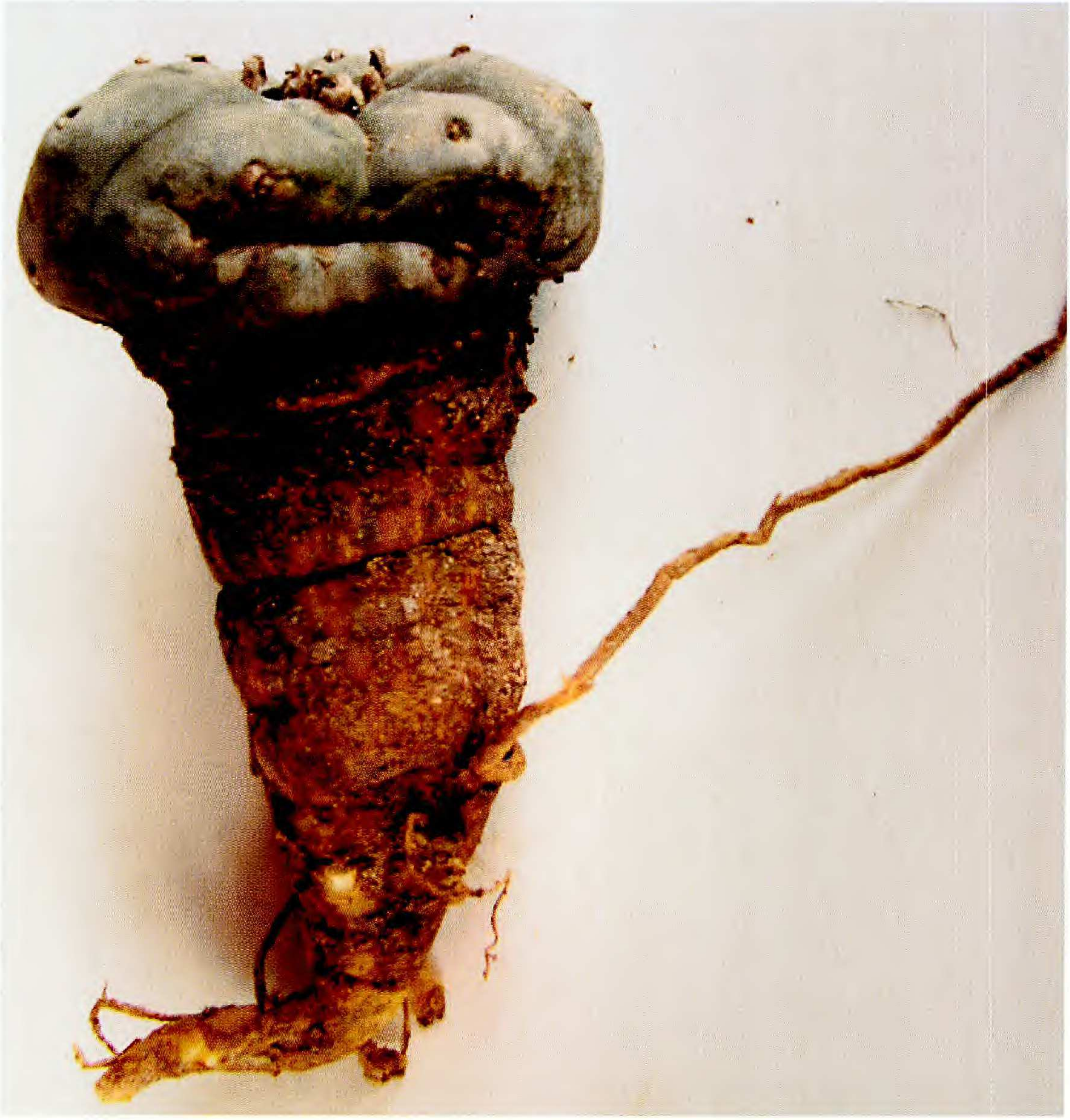

Fig. 5. This is a normal peyote plant with a single long lateral root and no obvious evidence of previous harvesting. The symmetry of the annular constrictions on the long subterranean stem suggests reduced growth rates in periods of winter and/or drought. The crown of this mature plant measures ca. 6 cm in diameter and has eight ribs. The shallow, elongated indentation in the side of the crown facing the reader is a scar from tissue sampling for DNA analysis 18 months previously.

the gravelly soil of their habitat and this would account for some reduction in the number of intact (and therefore observed) lateral roots.

Lateral roots emerged only from areas that were later shown to be taproot or hypocotyl, not from shoot tissue. But one plant examined in the field but not dissected and studied here had a root emerging from its side at about the same level as a lateral stem branch; that root might have emerged from the hypocotyl rather than the shoot but that is not known. This isolated field observa-

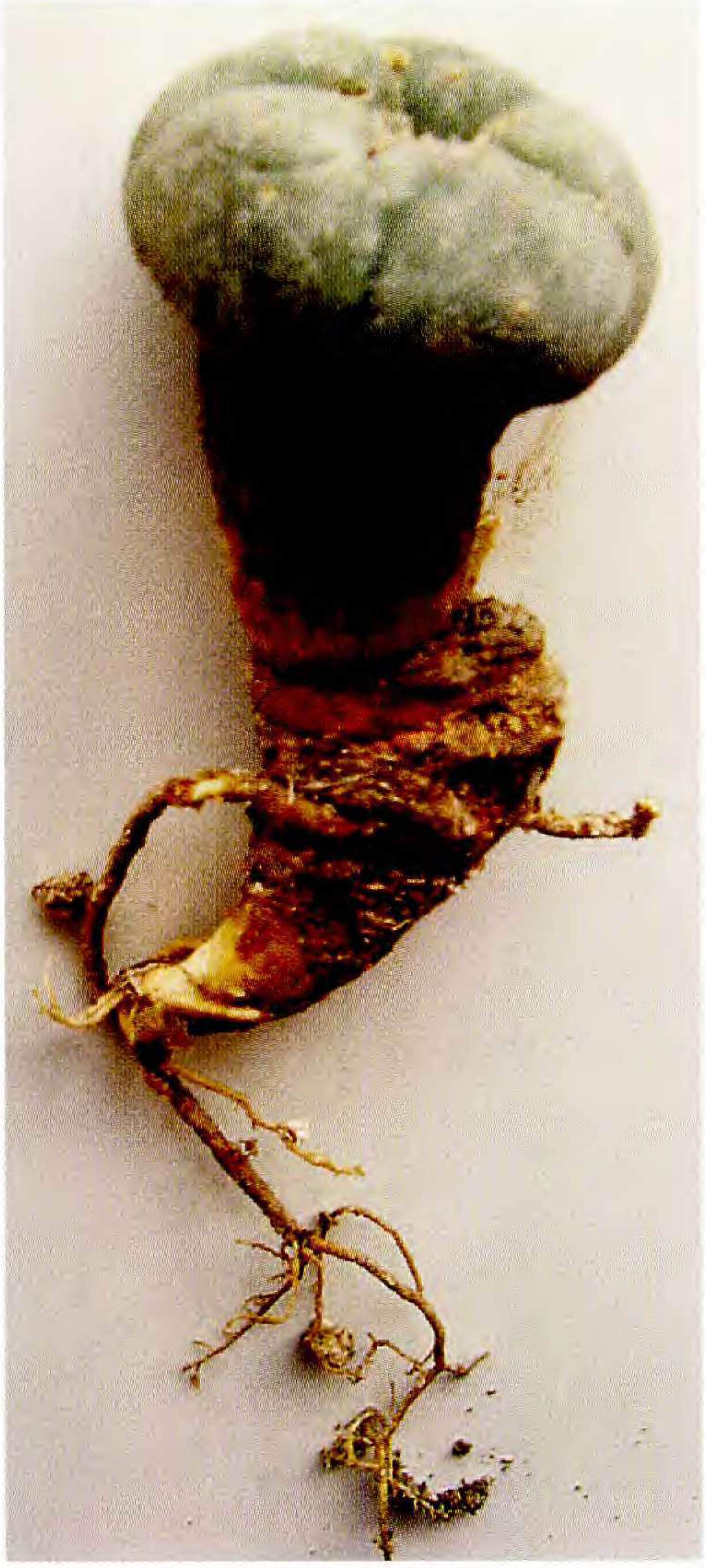

Fig. 6. This is a good example of a plant that was decapitated exactly once, several years ago, judging from the size of the new crown (ca. 5 cm diameter). Note that the crown-bearing lateral branch is markedly offset from the center of the original subterranean stem seen at the base of the lateral branch. That is a consequence of the fact that the lateral branch originated from an areole on the side of the subterranean stem.

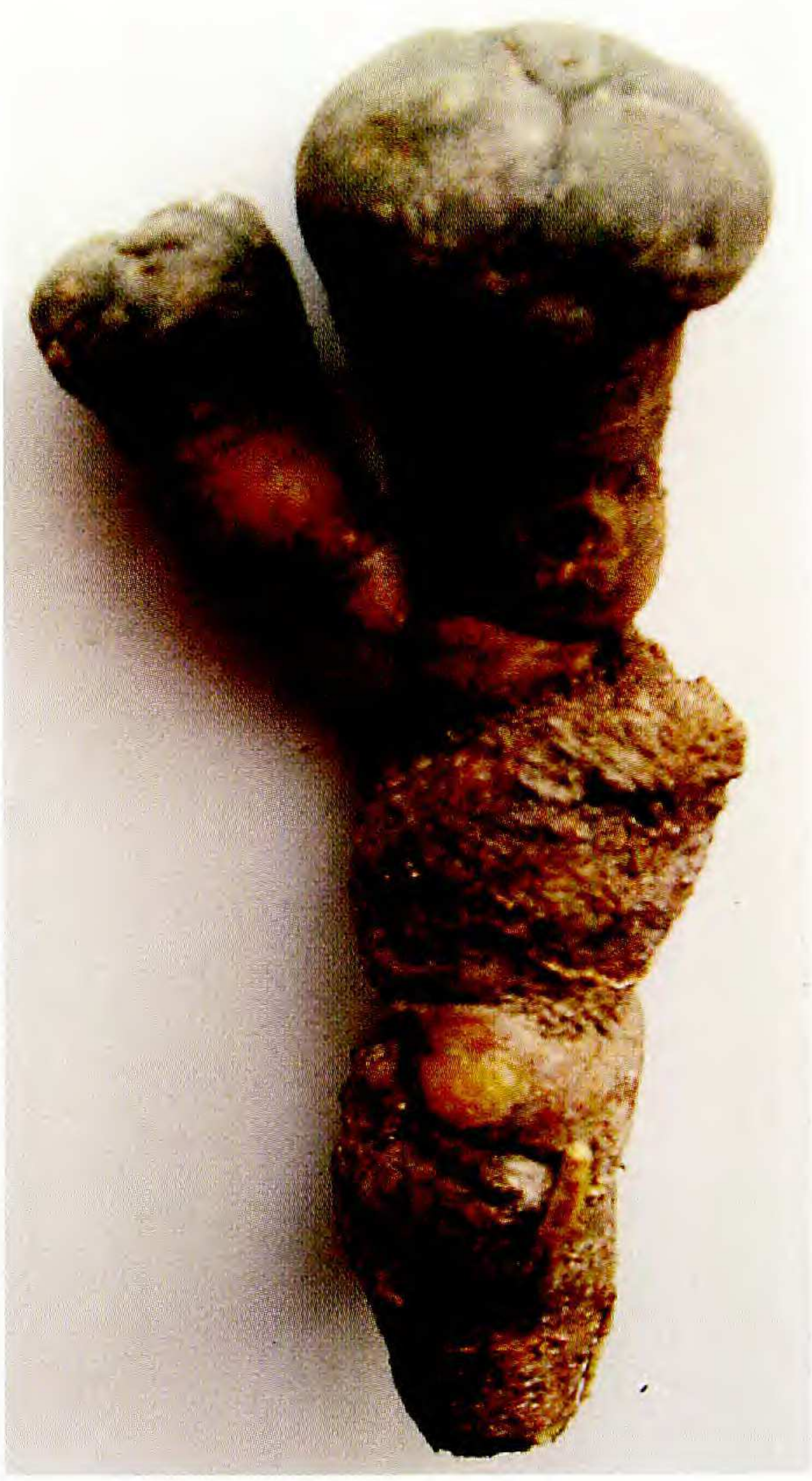

Fig. 7. This plant shows evidence of having been previously harvested at least twice. The small sizes of the two crowns on lateral branches from the original subterranean stem suggest that the most recent harvesting of this plant was perhaps 2–4 years ago. (The larger of the two crowns is about 2.5 cm in diameter. Both are 5-ribbed.)

tion should be interpreted in light of the possibility that some of what appear to be uppermost lateral roots of *Lophophora* may prove to be adventitious roots emerging from subterranean stem tissue. Whether ordinary subterranean stem tissue can produce adventitious roots, or whether adventitious roots can be produced only by regenerative lateral branches from a plant whose apical meristem has been removed, is an anatomical issue still to be resolved.

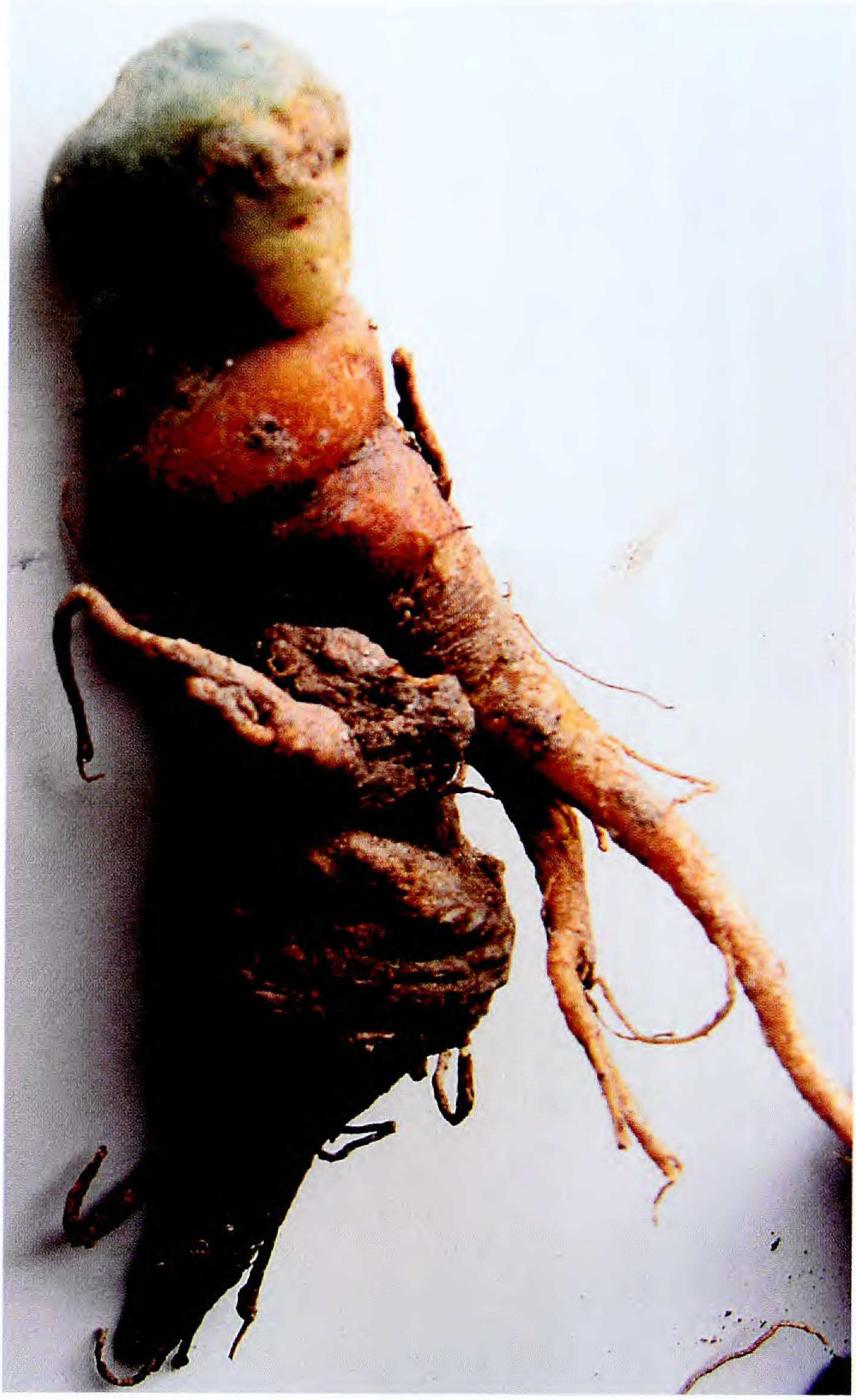

FIG. 8. This plant has been harvested several times. Only one of the two new crowns is visible from this view, but the two adventitious taproots, each originating in a stem branch bearing a crown, are both visible (protruding to lower right). Each of the two crown-bearing stems has recently developed by lateral budding from a *previous* harvest-associated lateral branch that was itself decapitated near ground level. The two new crowns, each with its own adventitious taproot, are well on their way to becoming independent of the parent rootstock. The original/parental plant, represented by its bark-covered taproot protruding to lower left from the original subterranean stem (also bark-covered), is degenerating, but its subterranean stem is still alive and still attached to the subterranean stems of the two clonal progeny.

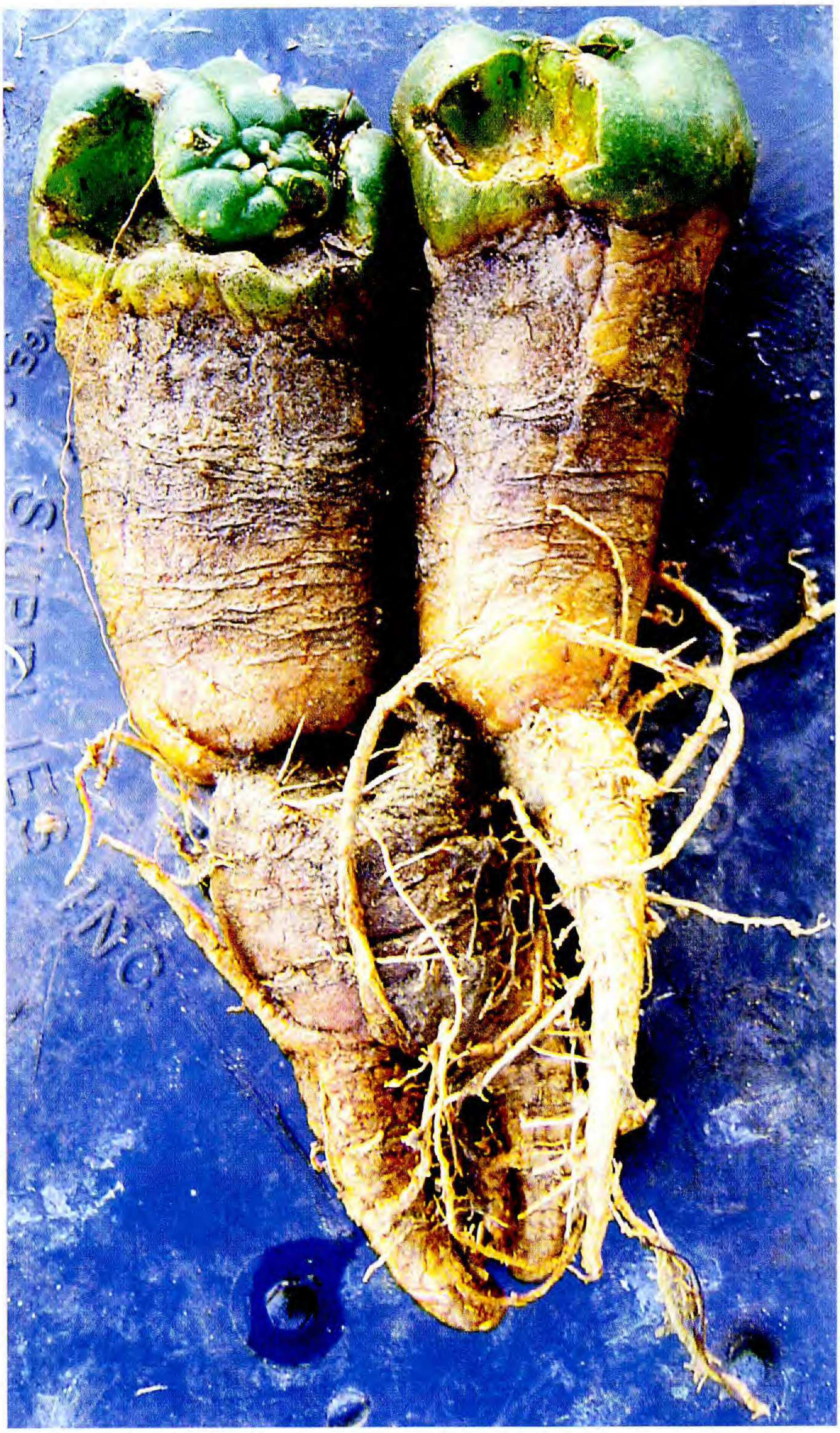

FIG. 9. Two vegetative clones that originated as lateral branches of the stem of a decapitated parent plant, which is degenerating but still clearly recognizable as the bark-covered subterranean stem with taproot extending down between and beyond the taproots of the two new plants. There is still a connection (not shown directly, but inferable from the inter-adherence of the plants in the photo) between the new plants and the parental plant, but each of the young plants has its own functional taproot and is virtually independent of the parental rootstock by now. Visible damage to the crowns is attributed to the activity of natural herbivores, probably terrestrial snails, which are known to consume *L. williamsii* crown tissue.

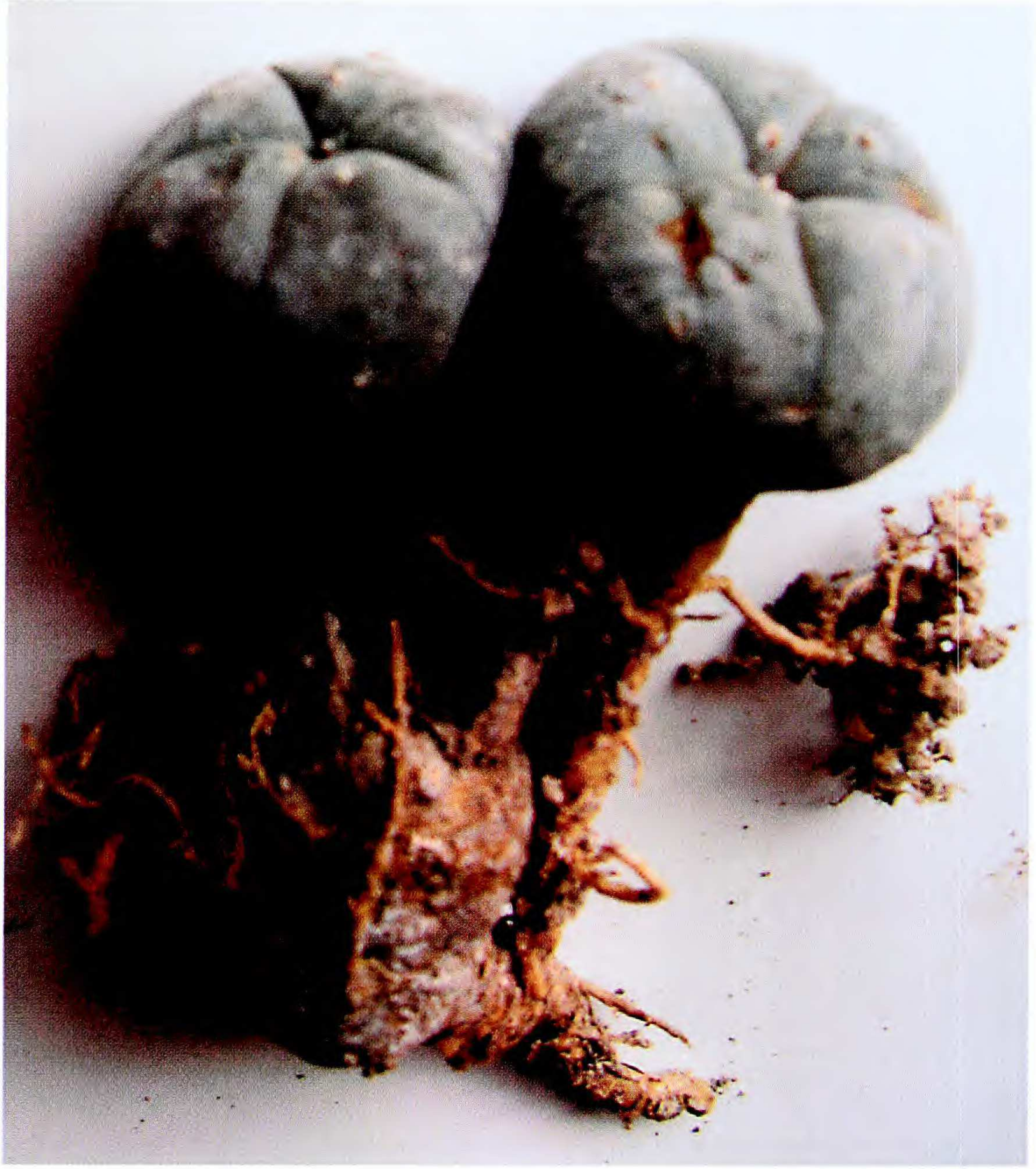

FIG. 10. This pair of "sister" vegetative clones is different from that shown in Fig. 9, in that the lateral branch on the left has effectively co-opted the taproot of the parent rootstock as its own taproot, whereas the lateral branch on the right has developed an adventitious taproot of its own, which will lead to its eventual separation from the parent rootstock.

Several plants had shrunken, withered tubercles on their subterranean portions. They were wrinkled and covered with bark just like all other subterranean portions, but their centers contained living parenchyma cells and an apical meristem, apparently the shoot apical meristem of the tubercle. The lowest one found on each adult plant was 15 mm below soil level on plant #1212, 30 mm on plant #1213, and 22 mm on plant #1214.

Withered tubercles were identifiable on subterranean, bark-covered por-

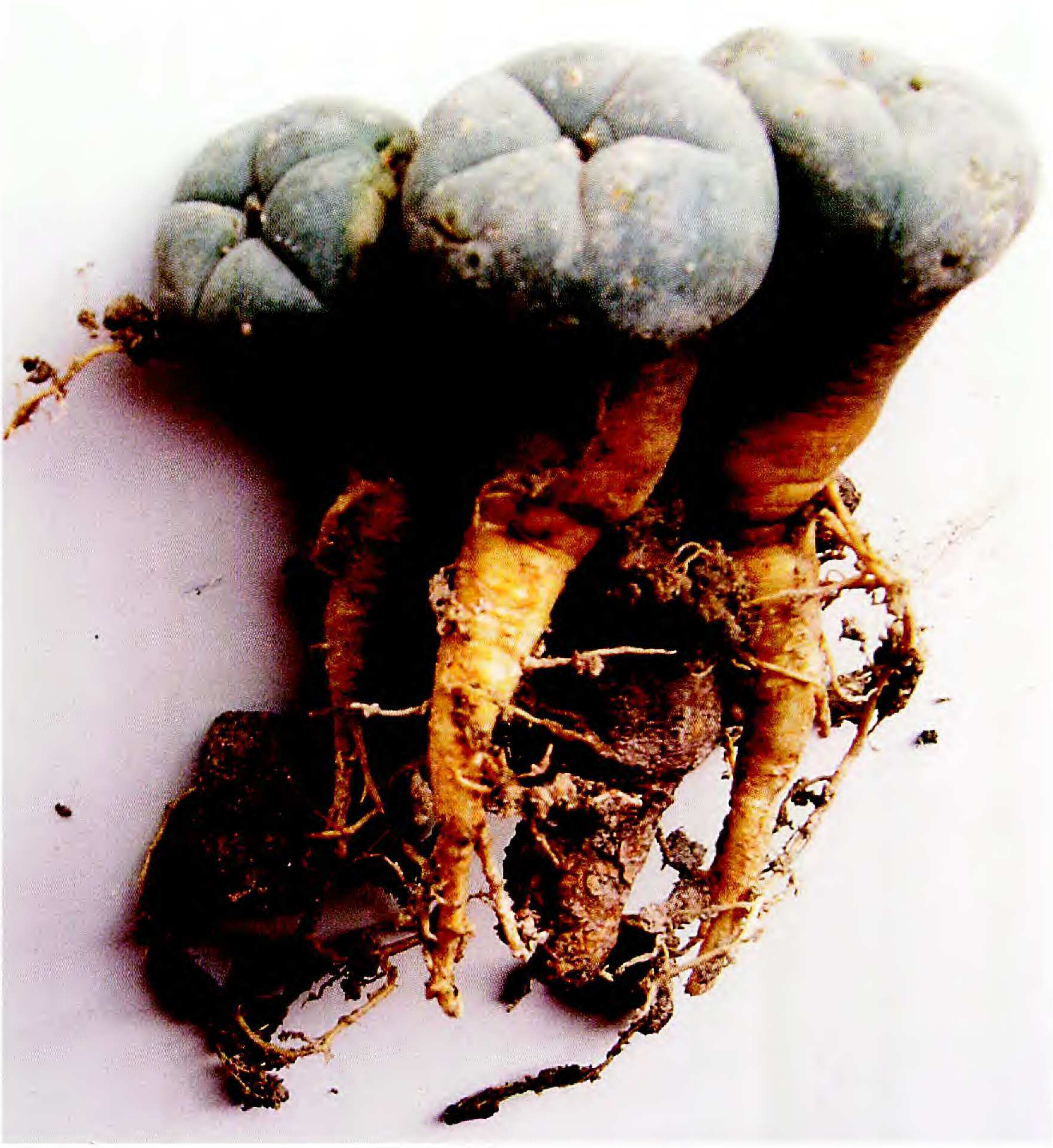

Fig. 11. Three clonal sister peyote plants—each anatomically and functionally on its way to independence with its individual crown and taproot—still attached to the parental subterranean stem (visible as the dark brown structure between the taproots of the middle and right clonal sister plants).

tions of two of the adult plants. Tubercles were reliably identifiable only in the uppermost 4.0 mm of subterranean shoot; below that, they had withered so much that the only visible remnants were peg-like structures 2.0–3.0 mm in diameter and 1.0–2.0 mm tall, which appeared to be deteriorated areolar tufts of trichomes. Bark was sufficiently wrinkled and rough that some of its irregularities resembled withered tubercles, making identification of tubercles difficult. Subterranean withered tubercles should have been aligned with the rows of tubercles on the aerial portions (Fig. 13), but there were only two cases in

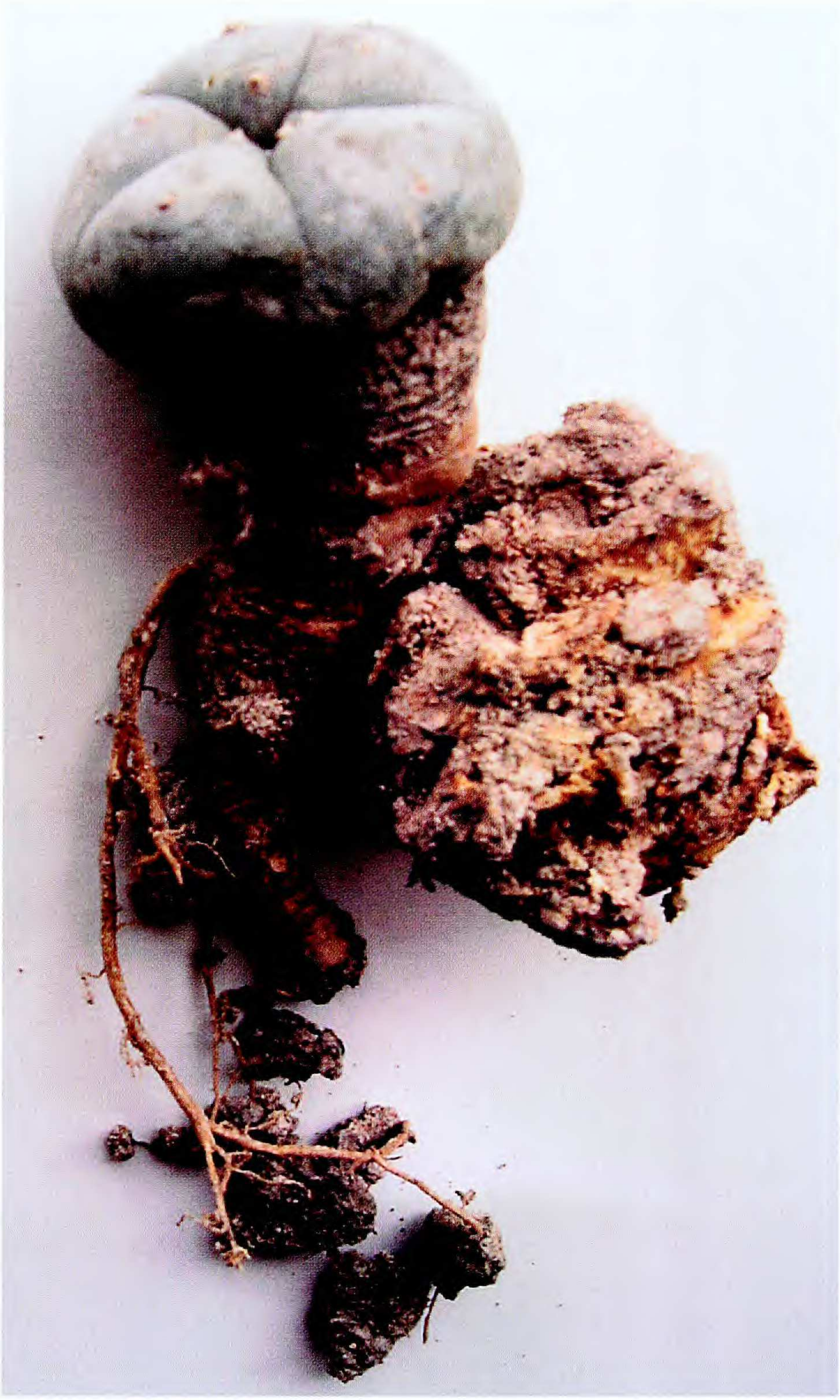

FIG. 12. The amorphous, decaying mass (right) that is tenuously attached to the subterranean stem of the live cactus (left), is the dead remnant of the subterranean stem of a parent plant that was decapitated by peyoteros sometime in the past. The young specimen that developed from the then-living parental subterranean stem, is now fully formed, with its own taproot, and is fully independent. The very low density of the attached mass suggests that it is in an advanced stage of decomposition.

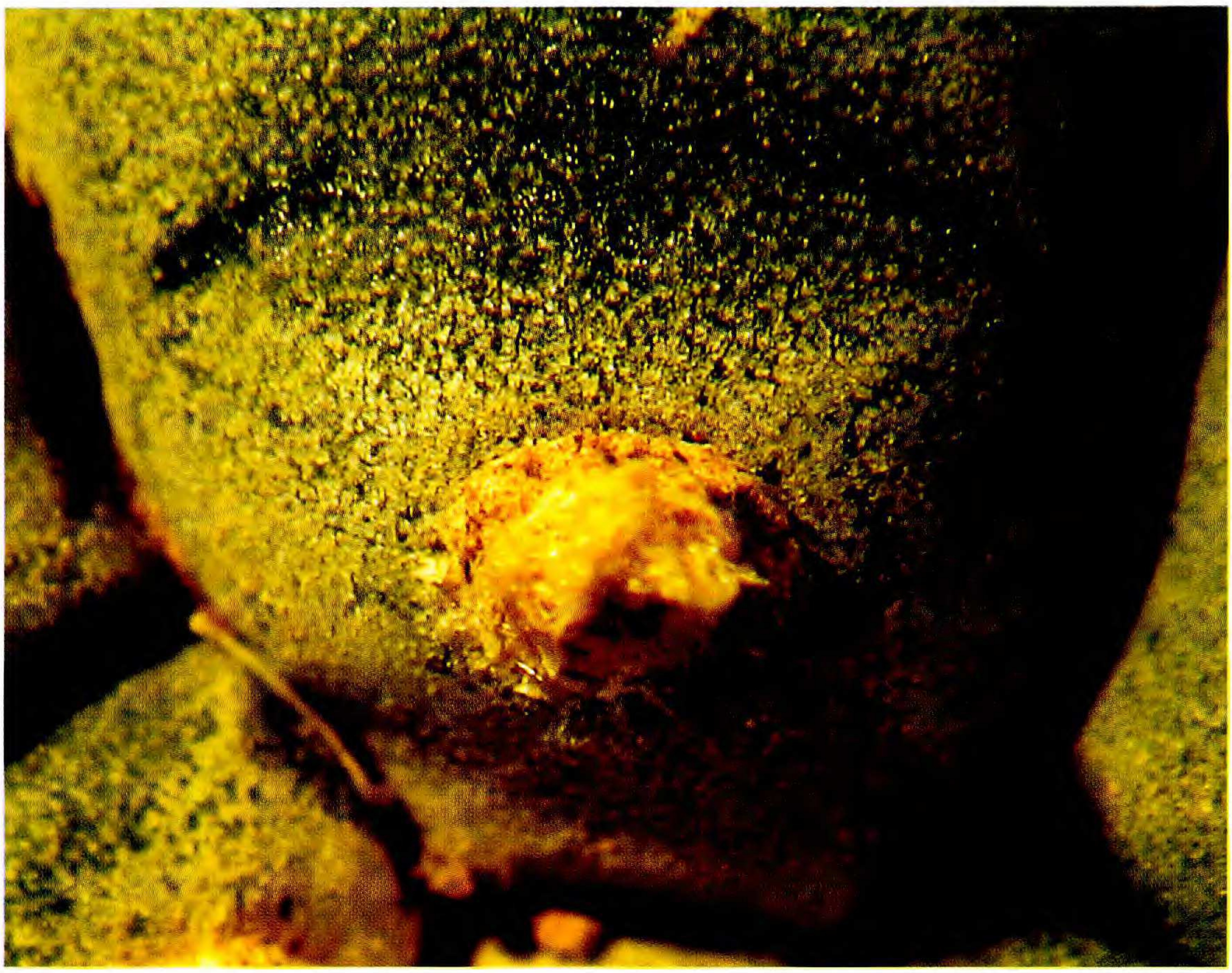

Fig. 13. *L. williamsii* young tubercle with areole bearing woolly trichomes. The apical meristem from which the areoles emerge radially is immediately below the field of the photograph.

which a row of withered tubercles could be identified by their areolar tufts (Fig. 14). We followed rows of tubercles from the aerial portions of shoots down into subterranean portions but usually could not find any identifiable withered tubercles, apparently the areolar tufts abscise from the plant.

Structure of the root

Very young regions of root (less than 0.5 mm in diameter) had an organization typical of most dicots. There was an epidermis, a thin cortex only a few cells thick, endodermis and vascular tissue consisting of small bundles of primary phloem alternating with arms of protoxylem, and metaxylem occupied the very center. An important point is that metaxylem contained significant amounts of living xylem parenchyma cells, it did not consist entirely of dead tracheary elements. This organization was found in only the two smallest, youngest plants, in samples taken from closest to root tips. Older portions of roots (2.5 to 30 mm in diameter) had altered their organization. They had lost their epidermis and cortex, and parenchyma cells in the metaxylem had begun to proliferate. Sec-

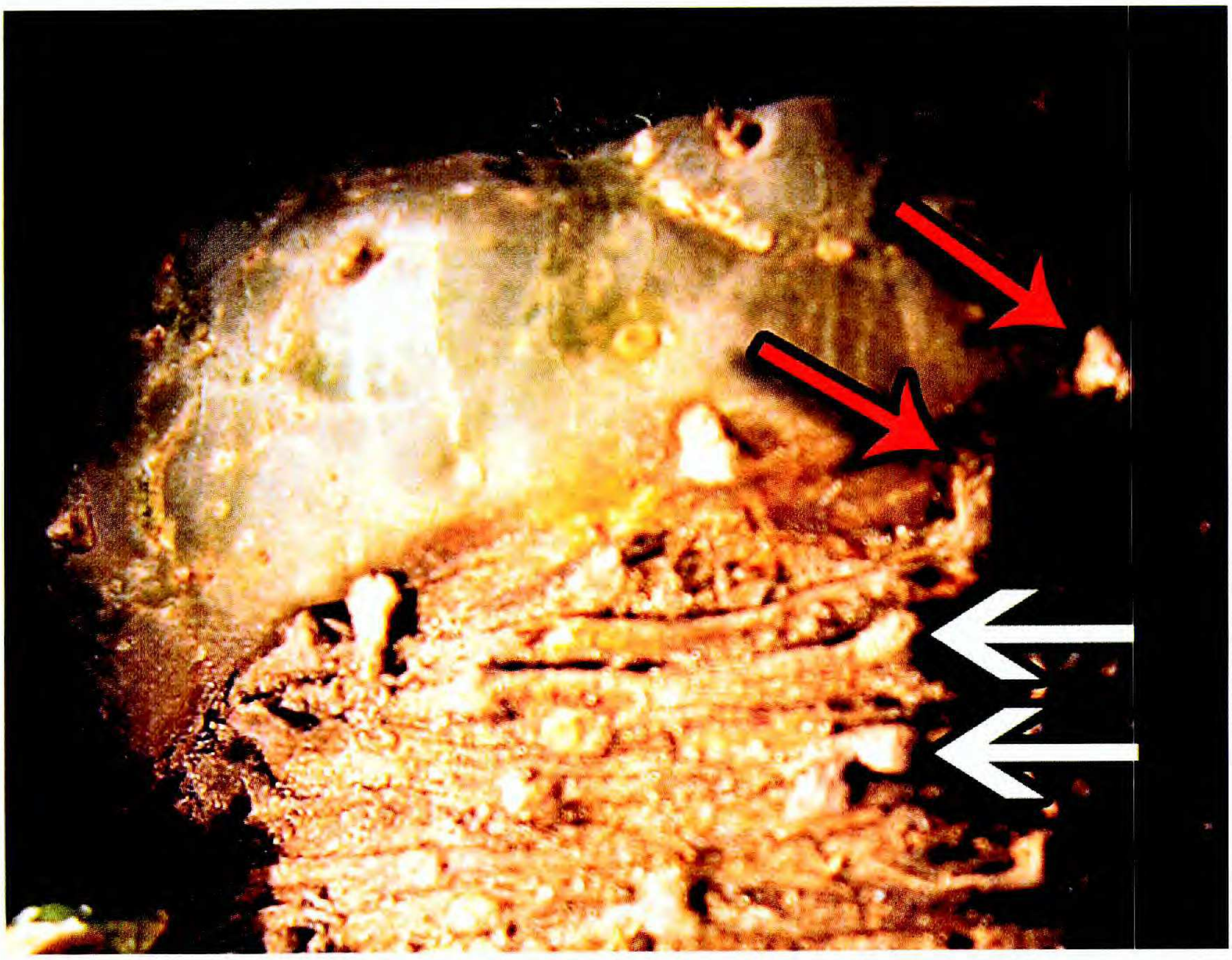

FIG. 14. *L. williamsii* tubercles with tufted areoles (red arrows) on lower portion of crown (green tissue in upper half of photo). On the corrugated brown surface of the subterranean stem (lower half of photo) in the transition between light and shadow are two lighter-colored protuberances, situated on a diagonal curve containing the two tufted areoles above in the crown. These protuberances (white arrows) are the visible areolar remnants of stem tubercles, which are the source of lateral branches of stem that develop in response to removal of the apical meristem.

ondary xylem (wood) and phloem were present, but epidermis, cortex and endodermis had been replaced by a bark consisting of thin flakes of cork. Just interior to the bark was a band of parenchyma that appeared to be cortex but which was really secondary phloem parenchyma. This cortex-like region was about 0.3 to 3.0 mm thick and extended inward from the bark almost to the vascular cambium. Thickness was correlated with root size: roots less than 4.0 mm in diameter had a cortex-like region only about 0.3 to 0.4 mm thick; roots about 10–12 mm in diameter had a cortex-like region 1.0 mm thick; and very large roots 30 mm in diameter had a cortex-like region 3.0 mm thick. The cortex-like region was recognizable as secondary phloem only because it had traces of collapsed sieve tube members in it. There were no vascular bundles in the cortex-like region other than very rare connections with lateral roots, and these were oriented vertically rather than radially or tangentially.

Metaxylem parenchyma proliferated in roots, producing such abundant amounts of parenchyma that the center of the root appeared to have a pith. Metaxylem vessel elements were pushed apart (such proliferation in an otherwise mature tissue is called dilatation), and parenchyma cells in the innermost, first-formed wood also underwent dilatation growth. This pith-like region could be identified as dilatated xylem (rather than true pith) by the presence of isolated vessel elements within it; these were easily visible with a handlens and dissecting microscope. In roots about 2.5 to 5.0 mm in diameter, the pith-like region was about 0.5 to 1.2 mm in diameter, but it was 3.4 mm in diameter in roots 12 mm wide, 10.0 mm in diameter in roots 22 mm wide, and 27 mm wide in roots 35 mm wide. Dilatation occurred in both the innermost, first-formed secondary xylem as well as the middle regions, but the outer regions of secondary xylem (the outermost 1.0–2.0 mm) had ordinary wood organization.

Root wood consisted of a ray system and an axial system (containing axially oriented cells such as vessel elements). Rays were extremely narrow, only 7.3 sd 2.3 µm wide and consisted of large, rounded parenchyma cells. The axial system consisted of vessel elements, paratracheal parenchyma in immediate contact with the vessels, and wide-band tracheids (WBTs). Wide-band tracheids are an unusual type of cell found in almost all cacti; they are short (range in *Lophophora*: 315 to 525 µm), broad spindle-shaped tracheids with secondary walls that are annular or helical (Mauseth et al. 1995; Mauseth 2004). There were no fibers in the wood. Just as rays were narrow, so were axial masses (98.4 sd 54 µm wide), and cross sections of root wood appeared to be rather solid when viewed with the naked eye or by dissecting microscope.

Structure of the shoot

Young regions of shoot differed from older regions by still having epidermis but lacking secondary xylem, phloem and bark. Epidermis was present on all aerial portions of shoots and had a blue-gray color. Hypodermis consisted of one layer of parenchyma cells. Both epidermis and hypodermis cells definitely did not have thickened walls so the shoot surface was very soft. Shoot cortex was always much thicker than the root's cortex-like region of secondary phloem. The thinnest cortex in an adult plant was 6 mm (in an old, below-ground portion of plant #1214) and the thickest was 32 mm at soil level in the same plant. Cortex was only 1.5 mm thick in the seedlings. The outermost cortex cells were columnar and aligned in palisades, the palisade cortex was about 3.5 mm thick. Cells of the inner cortex (located between the base of the palisade cortex and the phloem) consisted of large, rounded parenchyma cells. Cortical bundles were abundant throughout the inner cortex, extending to the base of the palisade cortex, and each bundle contained both xylem and phloem. Cortical bundles were easily visible by handlens and dissecting microscope.

A slender pith was present in the center of all stems. It was only 1.5 mm in diameter in seedlings, from 4 to 6 mm in plants of medium size, and 10 to 27 mm in diameter in the largest plant (i.e., plant #1214, which had the greatest girth). It consisted of just parenchyma cells with very rare spherical crystals and no mucilage. There were no medullary bundles at all and no dilated metaxylem. The lack of xylem in the pith was easily visible by handlens and dissecting microscope: with both of these, shoot pith looked very clean and homogeneous whereas the root's pith-like region was coarse and granular due to xylem in the dilatated region.

Young shoots had a ring of collateral vascular bundles located between pith and cortex, older shoots had secondary xylem and phloem as well. Secondary xylem in shoots was similar to root wood. Rays were narrow (149 sd 134 µm, just one or two cells wide) and consisted of parenchyma cells with no sclerification at all. The axial system consisted of small numbers of vessels and paratracheal parenchyma but large amounts of WBTs. As in roots, axial masses were narrow, only about 318 sd 179 µm wide. No xylem fibers were present in any sample. Due to the narrow rays and axial masses and the lack of fibers, shoot wood resembled root and the two could not be distinguished if a microscope view contained only wood and no other tissues. Secondary phloem in shoots did not produce a cortex-like region as it did in roots; instead, as the sieve tube members stopped conducting, phloem collapsed into a thin, tangential band.

All subterranean portions of *Lophophora* shoots were covered by bark similar to that on older portions of roots. An unusual feature was that shoot bark occasionally contained crystals and vascular bundles, indicating that the cork cambium had arisen deeply enough in the shoot cortex to cut across cortical bundles; however, both crystals and vascular bundles were too small to be visible with a handlens examination of bark.

Structure of the hypocotyl

The hypocotyl is the short (less than 10 mm long) region located between the seedling shoot (epicotyl) and the seedling root. The structure of the hypocotyl in *L. williamsii* had characters of both the root and shoot. The center of all hypocotyls was root-like because it consisted of dilatated metaxylem and innermost secondary xylem, so it too was pith-like. It could be identified as not being a true pith by the presence of vessel elements and WBTs interspersed with the parenchyma cells. The outermost regions were true cortex, and even though a hypocotyl is not a part of the shoot, the hypocotyls of *Lophophora* had cortical bundles. Hypocotyl cortex width was wider than that of the cortex-like region in roots, narrower than the true cortex of shoots in each plant. All hypocotyl samples had abundant secondary xylem and phloem, which was similar to that in both roots and shoots. Hypocotyl bark was similar to that of shoots, having occasional bits of cortical bundle that had been cut off by a cork cambium located deep within the cortex.

TABLE 2. Distinguishing characters of shoots and roots of *L. williamsii*.

	Shoots	**Roots**
Outer tissues	True cortex; appears granular due to presence of cortical bundles. At least 5 mm or more thick.	Cortex-like region; appears smooth due to lack of cortical bundles. At most only 3 mm thick.
Center	True pith; appears smooth due to lack of medullary bundles and lack of dilatated metaxylem. Width is not a reliable criterion.	Pith-like region; appears granular due to dilatated metaxylem and innermost secondary xylem.
Withered tubercles	Sometimes present, not always easy to identify if bark is rough.	Never present.
Lateral roots	Never present on shoots? May be confused with post-harvest adventitious roots.	Common on taproots, but could be absent.

DISCUSSION

This study shows that roots and shoots of *L. williamsii* differ significantly in several features. At least in fresh plants, the two organs can be distinguished easily and reliably using just a handlens or dissecting microscope (Table 2). Both root and shoot have an outer region that resembles cortex, but the true cortex of shoots has a granular appearance because it contains numerous cortical bundles (Fig. 15), as is true of many cacti (Sajeva & Mauseth 1991; Mauseth & Sajeva 1992). In contrast, the outer region of roots resembles cortex but is in fact an accumulation of secondary phloem, which has a very smooth appearance as seen with a handlens. The vascular bundles of lateral roots pass through this cortex-like region of secondary phloem, but because lateral roots are so sparse and because their vascular bundles are oriented vertically, there is little chance of confusing the shoot and root outer tissues. Roots of other cacti also have this outermost cortex-like region (Stone-Palmquist & Mauseth 2002). The true cortex of the root of *L. williamsii* is pure parenchyma (Fig. 16) and does not contain secondary phloem.

The center of shoots is occupied by true pith, which is homogeneous in appearance due to the lack of medullary bundles in *Lophophora williamsii* (Fig. 17). Medullary bundles are common in many species of cacti but lacking in others (Mauseth 1993). In the roots of most species, metaxylem either has no parenchyma or if it does, the parenchyma does not undergo proliferation, so that roots of most plants have no pith-like region at all and can easily be distinguished from shoots (Mauseth 1988). The pith-like region of roots in *L. williamsii* (Fig. 18) makes the roots look like shoots at first glance or with just the naked eye, but because the pith-like region in cacti originates by cell division in root metaxylem (Gibson 1978; Loza-Cornejo & Terrazas 1996), it has a granular appearance when examined with a handlens.

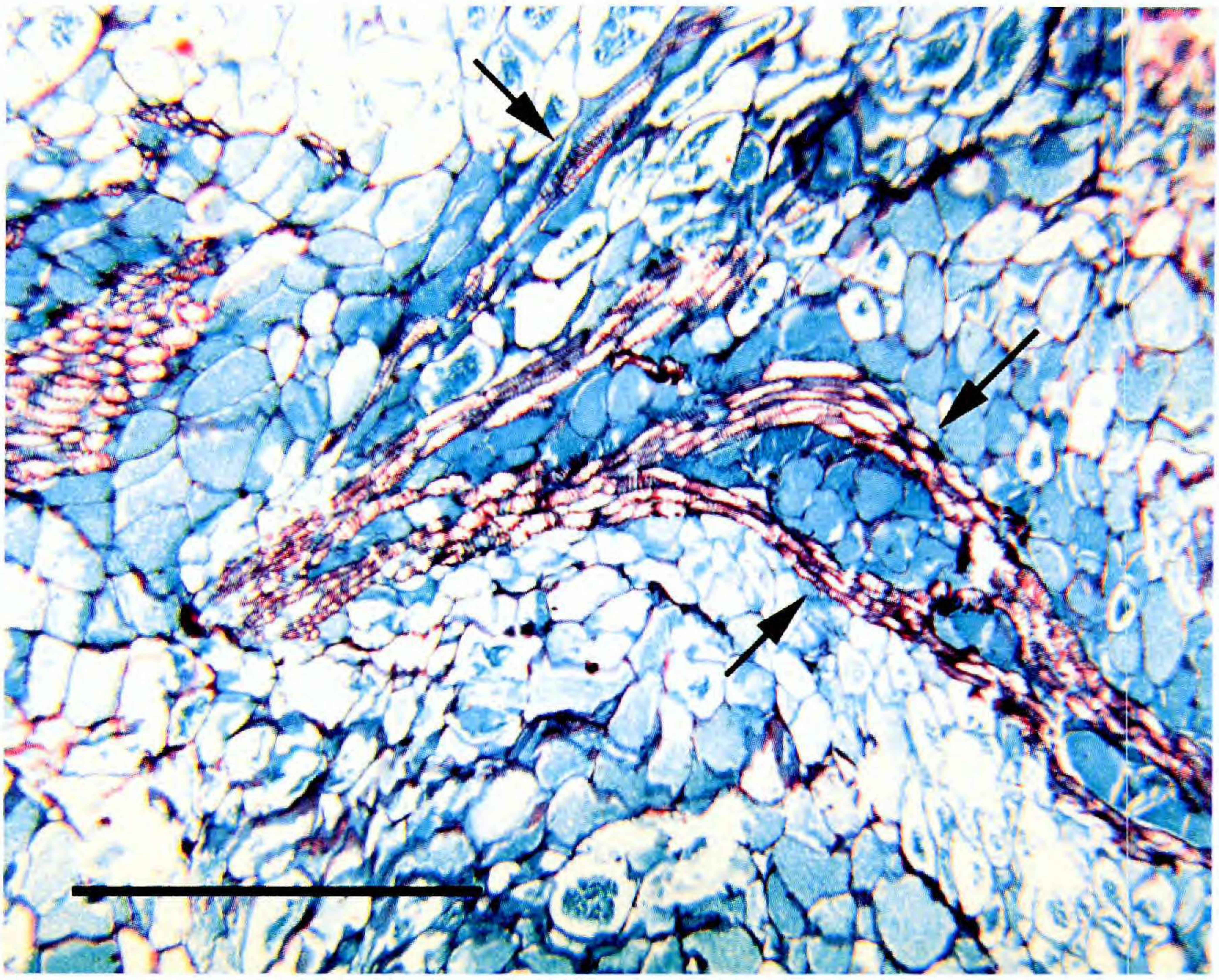

Fig. 15. Cortex of shoot of *L. williamsii*. Arrows indicate cortical bundles running through the parenchyma. All visible parenchyma cells are cortex parenchyma. Scale bar (lower left) = 1 mm.

Two characters might be useful for distinguishing shoots from roots without cutting plants open to examine the cortex-like regions and pith-like regions. Lateral roots emerged from the sides of other roots and from the sides of hypocotyls, but none was seen on any part of the three adult shoots we examined. It is possible that shoots had produced adventitious roots which had either broken off when the plants were collected or which had abscised before collection. No remnants of such roots were seen when we examined the sides of subterranean portions of shoots with a dissecting microscope, but these plant parts were so wrinkled and bark-covered that we could have missed any that were present. However, as we examined the microscope slides of subterranean portions of shoots, we did not encounter any vascular bundles that would have indicated adventitious roots had been present. One plant, examined in the field, had a root emerging from its side at about the same level as a lateral branch; if that root was emerging from the shoot rather than the hypocotyl then shoots as well as roots might bear roots. Now that anatomical characters can be used to distinguish roots from shoots, it will be possible to examine more plants in the

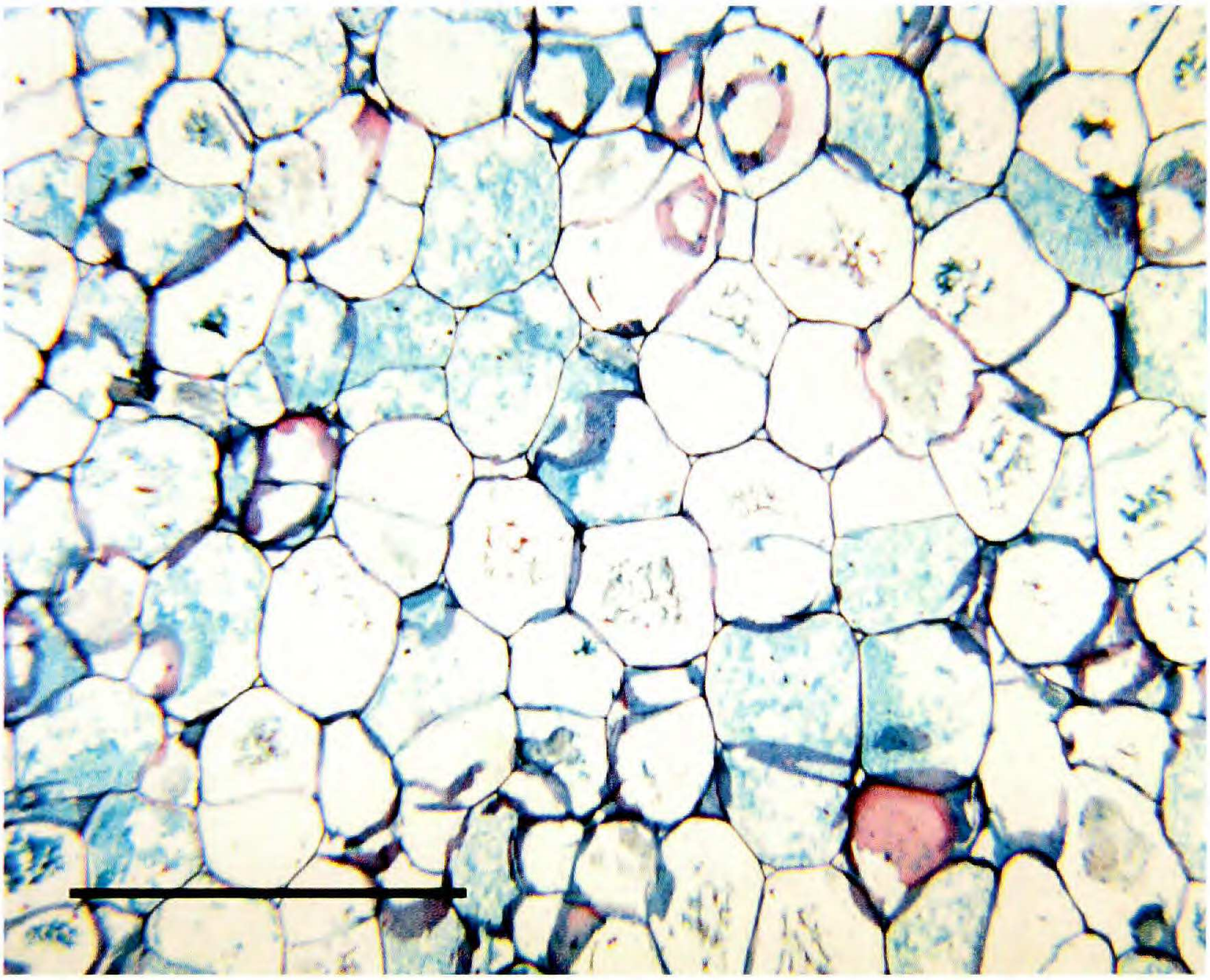

Fig. 16. Cortex of root of *L. williamsii*. All cells are cortical parenchyma cells. Absence of cortical bundles is conspicuous. Scale bar = 1 mm.

field to see how frequently shoots bear roots from their sides, and the extent to which such adventitious root development is associated with branching of subterranean stem tissue in response to peyote harvesting or removal of the apical meristem by natural processes.

If the below-ground portion of the plant has withered tubercles, it must be part of the shoot rather than root or hypocotyl. However, we did not find any withered tubercles on one of our adult specimens despite a search with a dissecting microscope. Apparently they either wither so much that they become unrecognizable or they abscise, so their absence cannot be used as proof that the structure is either hypocotyl or root. When trying to find withered tubercles in the field, one should search in a line continuous with the line formed by the rows of tubercles in the aerial shoot, because all tubercles are formed in rows (just as on the ribs of columnar cacti: Mauseth 2000).

If a plant remnant is to sprout and continue growing after its top has been harvested, the presence and health of these withered tubercles is important. If a plant is harvested by being cut too low, only root or hypocotyl will remain in

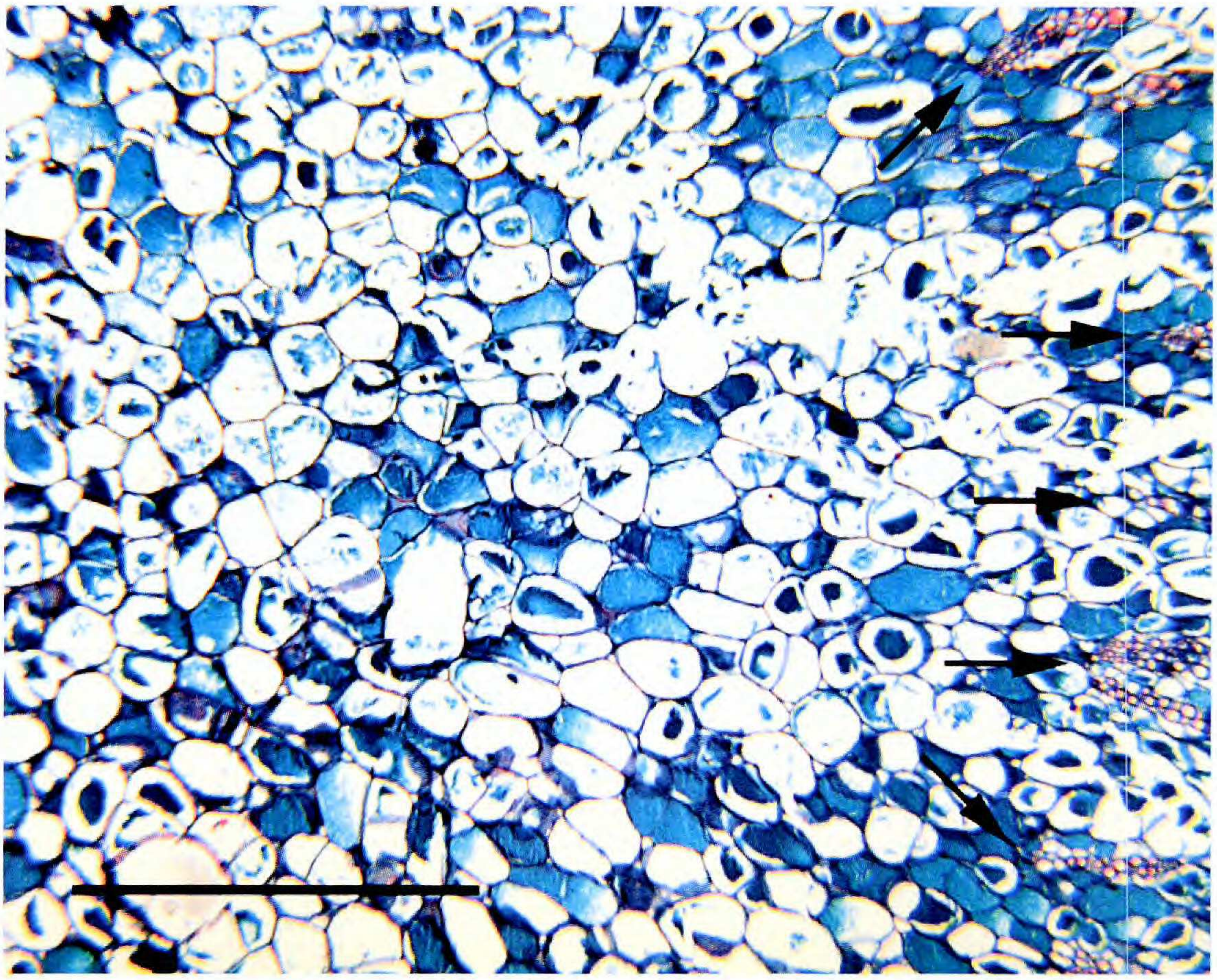

Fig. 17. Pith of center of shoot of *L. williamsii*, showing pith as pure parenchyma without xylem (center and left). Arrows (right) indicate the innermost primary xylem of the vascular bundles in the ring of bundles at the perimeter of the pith. Only part of the ring of vascular bundles is shown (right), and all the cells on the left are true pith. Scale bar = 1 mm.

the ground and neither of these have axillary buds, so neither can produce a bud to replace the harvested shoot. If the plant is harvested by being cut through the subterranean shoot, and if the remaining portion of shoot has healthy tubercles—withered but with an axillary bud—then the remaining portion should be able to sprout and grow and be ready for harvesting again in a few years. But if the remaining shoot has abscised all its tubercles, or if they have withered so much that they are no longer healthy, then the remaining piece of plant will not be able to sprout and will instead eventually die for lack of photosynthetic tissues. Tubercles located higher on the subterranean portion of the shoot are younger and presumably healthier than those lower down, deeper in the soil and closer to the root. If plants are harvested by cutting the subterranean shoot rather high—closer to soil level—the greater the chances are that the residual piece of plant will have healthy tubercles and will be able to re-sprout.

With the information discovered in the current study, we now have the tools to examine in detail the potential for regrowth after different types of harvest-

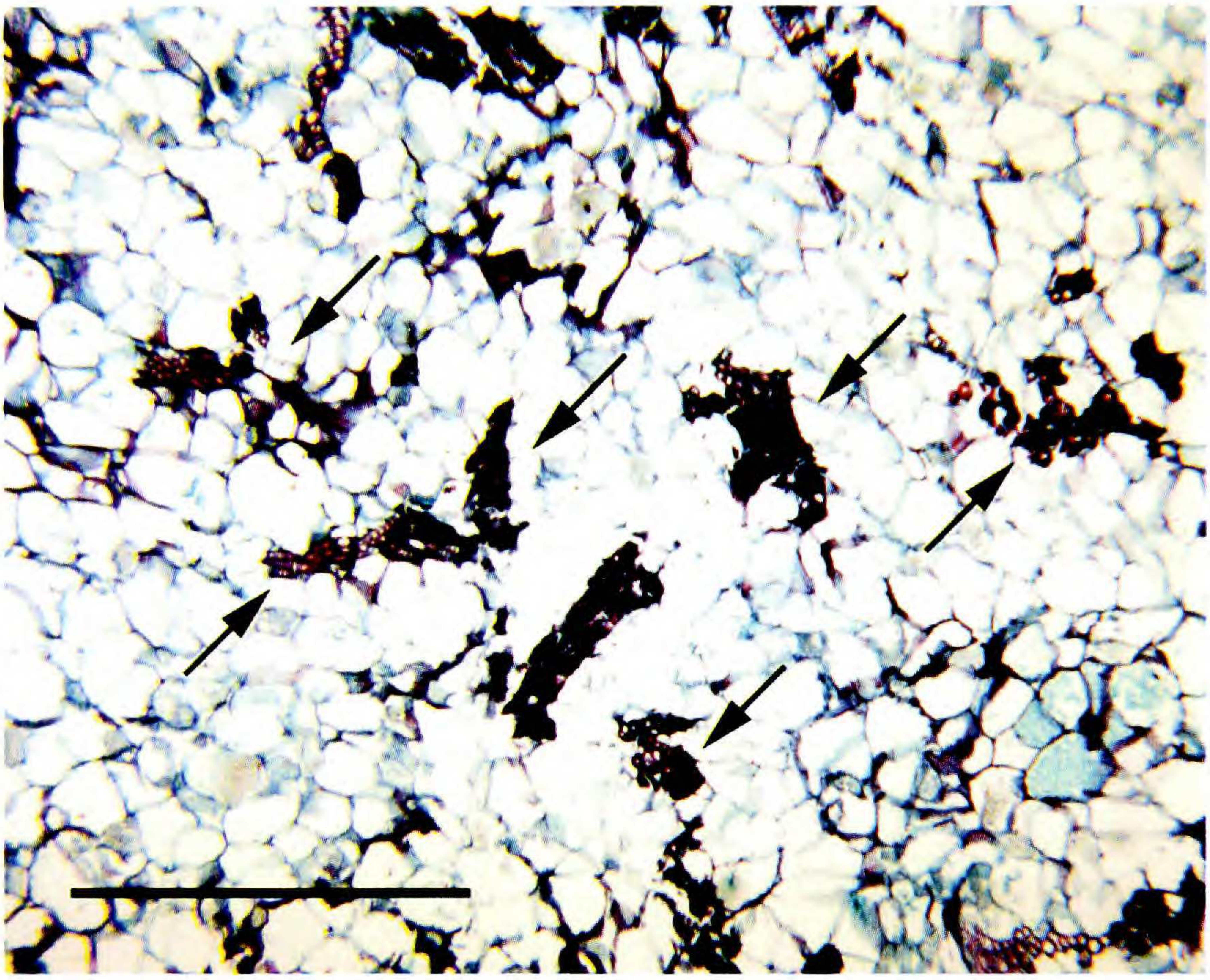

FIG. 18. Dilatated metaxylem of center of root of *L. williamsii*. All arrows indicate masses of dark-staining metaxylem tracheary elements. All parenchyma cells in the image are metaxylem parenchyma cells, not pith. Scale bar = 1 mm.

ing. A set of plants could be harvested at various depths below soil level with the certainty that all had been cut high enough that some shoot tissue had been left on the remaining plant portion. The harvested top of each could be examined for withered tubercles. Presumably if plants are cut so high that their harvested tops have several recognizable withered tubercles near the cut, then the remaining portion also has at least a few tubercles and will sprout. But if plants are cut so low that the harvested tops have few or no identifiable withered tubercles near the cut—and if the cut does pass through shoot tissue and not root tissue—then probably the remaining shoot also has few or no tubercles capable of sprouting. It is even possible to try cutting the plants through the hypocotyl to see if it is capable of forming adventitious shoot buds; that capacity is rare in hypocotyls but is known to occur in a few species.

Preliminary data on plants under greenhouse conditions were collected on 11 plants over a period of three years (not synchronically). Six plants were cut low (approximately 1.5 crown diameters below the base of the crown, and in every case at least 50 mm below the base of the crown). The distal subterranean portions of those plants were then observed for at least eight months. No new stem branches were observed on any of the six low-cut plants. The other five plants were cut high (at about the base of the crown, and in no case more than 5 mm below the base of the crown) and similarly observed. One or more crown-bearing lateral branches from the decapitated subterranean stem were observed within five months on three of the five plants, and within eight months on all five high-cut plants.

Future studies include a similar greenhouse experiment on regrowth, with substantially larger numbers of plants and with varying measured depths of cut expressed as a fraction (or multiple) of crown diameter, so that crown diameter could be used as a practical guide for harvesting peyote in the field. It will be noted whether the cut goes through root, stem, or hypocotyl. That titration of the effect of depth of cut on regrowth in the greenhouse will be followed by a similar experiment conducted in the field, with individually identified and permanently marked plants. It is anticipated that results in the field may differ from greenhouse results, due to harsher environmental conditions in the field and the possibility that some of the smaller plants in the field may have been harvested previously, perhaps leaving less than the critical mass of subterranean stem tissue needed to regenerate viable photosynthetic stem tissue. These factors may affect regeneration and survivorship in all experimental groups.

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